



IMPERIAL INSTITUTE
OF
AGRICULTURAL RESEARCH, PUSA.

PUBLISHED
SEPTEMBER, OCTOBER, NOVEMBER, DECEMBER, 1931

COMPOSED AND PRINTED BY THE UNIVERSITY OF CHICAGO
PRESS, CHICAGO, ILLINOIS, U S A.

THE
BOTANICAL GAZETTE

EDITOR
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VOLUME XCII
SEPTEMBER-DECEMBER 1931

WITH SIX PLATES AND THREE HUNDRED THIRTY-TWO FIGURES



THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

THE CAMBRIDGE UNIVERSITY PRESS, LONDON
THE MARUZEN COMPANY, LIMITED, TOKYO
THE COMMERCIAL PRESS, LIMITED, SHANGHAI

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DATES OF PUBLICATION

No. 1, September 17; No. 2, October 21; No. 3, November 14; No. 4, Decem-
ber 21

ERRATA

VOL. XCII

P. 262, for citation 8 read 12
for citation 9 read 8
for citation 10 read 9
for citation 11 read 10
for citation 12 read 11

THE
BOTANICAL GAZETTE

September 1931

THE GAMETOPHYTES OF THREE SPECIES
OF EUISETUM

ELDA R. WALKER

(WITH FIFTY-FOUR FIGURES)

Introduction

A few years ago the writer (9) reported the occurrence of gametophytes of *Equisetum laevigatum* A. Br. in several localities, and the growing to maturity in the greenhouse of the gametophytes of *E. arvense* L. and *E. telmateia* Ehrh. Since that time the prothallia of these species, as well as those of *E. kansanum* Schaffner, have been found in great abundance in their native habitats. All have also been grown in cultures in a way that made observation possible without injuring them; consequently the same individuals could be repeatedly studied over an extended period. The results of observations on both the wild gametophytes and those grown in culture are here recorded.

Material and methods

Gametophytes of *E. kansanum* have been found on various creek banks in the vicinity of Lincoln, Nebraska, at intervals from 1921 to the present. As the gametophytes of this species cannot be distinguished from those of its close relative *E. laevigatum*, identification of wild specimens depends entirely upon the species growing in the vicinity. Because of this there may be some confusion as to plants taken from nature. All results obtained in all cases were checked by gametophytes grown in culture from spores of known identity.

In the summer of 1922 gametophytes of *E. telmateia* were found in abundance, as follows: at Longbranch, Washington; near the Puget Sound Biological Station at Friday Harbor, Washington; at Olga, Washington; and near Forest Grove, Oregon. At only one place, near Weeping Water, Nebraska, have gametophytes of *E. arvense* been secured from the natural habitat. They were, however, abundant there.

Cultures on sphagnum of *E. kansanum* grew continuously more than two years, those of *E. telmateia* about the same length of time and those of *E. arvense* eight months. All three species were also grown to maturity on soil, but most of the results here recorded were from cultures on sphagnum. The plants from these cultures agreed in all essentials with those grown on soil and those found in their native habitat. Freedom from soil particles simplified both the observations on living specimens and the making of paraffin sections.

The culture method used was a slight modification of that employed by HARTMAN (6) in her studies of fern antheridia.¹ Sphagnum was boiled for 45 minutes, and then packed tightly in boiled moist chambers to a depth of 1-2 inches. All surplus water was pressed out. The boiling was only to kill any spores or seeds present, no attempt being made to secure completely sterile cultures. The dishes were covered until cooled, when the spores were shaken directly from the cones on to the surface of the sphagnum by tapping with the finger. The dishes were again covered and placed on the sill of a north window. As no attempt was made to sterilize or wash the spores, fungi and algae invariably appeared. Often fungi started growth with or even before germination of the spores of *Equisetum*. The former were controlled as they appeared with a solution of potassium permanganate. Strong and weak solutions gave equally good results, hence no attempt was made to standardize the solution. Enough crystals of potassium permanganate were added to distilled water to make a deep purple solution. This was poured over the infected areas freely and excess liquid removed. In cases of bad infection the entire culture was submerged in the solution and allowed to stand 5-10 minutes. Then the dish was drained as completely as possible. This treatment was harmless to the gametophytes; in fact,

¹ This method gives equally good results in growing sphagnum from spores.

it seemed of benefit, as well as destroying the fungi. Even cultures not infected with fungi that became sluggish renewed their activity after the addition of potassium permanganate.

As the dishes were kept covered the cultures rarely needed watering. If they became dry, however, they were moistened either with distilled water or with potassium permanganate solution.

Algae occurred in nearly all cultures. At first attempts were made to control them by treating with a solution of copper sulphate, but it was found that cultures containing algae grew better than those without them, so no further control was attempted. BUCHTEIN (3) also found algae harmless to cultures of *Equisetum*. It is possible that they fix nitrogen, as described by ALLISON and MORRIS (1) for certain blue-green algae. While both blue-green and green algae occurred, there was no evident difference in the effects upon the cultures.

The gametophytes in cultures grew normally and produced numerous antheridia, archegonia, and sporophytes. Only in two respects did they differ from gametophytes found in nature. They were slightly more slender and of a little lighter color. During the first year growth was largely in one direction, toward the window, thus producing long narrow thalli. This, however, was an advantage in the observation of sex organs. Eventually in all cases the zone of meristem gradually widened until the gametophytes took a form similar to those found out of doors and described by the writer (9). Often the meristem grew more rapidly at some points than at others, and the thalli came to appear as if composed of radiating lobes.

At frequent intervals specimens were fixed and paraffin sections made for verification of observations on living plants. Formalin-acetic-alcohol (50 per cent alcohol 93 cc., glacial acetic acid 1 cc., neutral formalin 6 cc.) gave the best results, although chromo-acetic and chromo-acetic-osmic solutions were also used.

The use of moist chambers as culture dishes made it possible to examine the thalli with lenses of sufficient power so that archegonia and antheridia could be recognized without removing the plants from the substratum, or in any way injuring them. Thus the same thalli were repeatedly observed. The same individuals were observed at frequent intervals during the period of two years (*E. kan-sanum*), and many thalli of all species were studied through shorter

periods. Because of the tendency, during the earlier stages, to grow toward the light, entire thalli could be removed and examined as thick sections. After examination, such thalli were fixed and observations were checked in paraffin sections

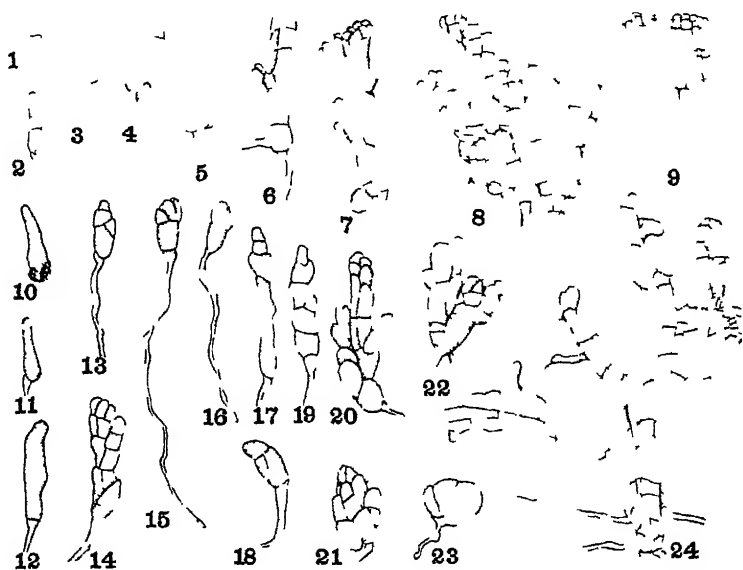
The plants from which the spores used in these studies were taken were identified by Dr. J. H. SCHAFFNER.

EQUISETUM KANSANUM

Gametophytes of this species, as was the case with *E. laevigatum* previously described by the writer (9), have never been found in close proximity to the adult sporophytes. They occur on the narrow floodplain of streams and ponds, whence the spores have been washed from plants growing at higher levels on the prairies. As both *E. laevigatum* and *E. kansanum* commonly occur in Nebraska, and as their gametophytes grown in culture cannot be distinguished, it was inferred that gametophytes found in a vicinity where *E. kansanum* predominates are probably of that species. For this reason the following discussion will deal with gametophytes grown from spores known to be *E. kansanum*. Everything said of this species may correctly be considered as applying equally to *E. laevigatum* previously discussed by the writer (9). They occur in the same type of habitat, have the same peculiar brownish green color, the same development, and the same ultimate form. *E. kansanum* is described because it was the species used in the more extensive studies

As observed by BUCHTEIN (3) and CAMPBELL (4) for other species of *Equisetum*, the first division of the spore results in a smaller cell, which gives rise to the first rhizoid and to a larger cell from which the thallus is developed. The next division, as well as succeeding ones, may occur in any plane and in any order (figs. 1-9). In some cases, especially in dim light, a filament (figs. 1, 6) of several cells may develop. In such cases further development of the gametophyte takes place from division of the end cells of the filament. More commonly the second division occurs in a different plane from the first (figs. 2, 4, 5, 7, 9), and a massive tissue at once begins to form. After only a few divisions the two parts of the thallus become differentiated, a massive basal region with little or no chlorophyll and upright green branches of characteristic form (figs. 2, 5-9). Early

from the massive base is developed a region of meristem (fig. 8a), which continues the growth of the base and gives rise to additional upright green branches, archegonia and antheridia. The meristem widens as it grows, it also grows more rapidly at some points than at others. This causes the thallus to become more or less lobed, and to develop early a tendency to radial form, as described by KASHYAP (7, 8) for *E. debile*



FIGS 1-24.—FIGS 1-9, early stages in development of gametophytes of *E. pan-sanum*, $\times 30$. FIGS 10-24, same of *E. arvense* (fig. 24 with two antheridia), $\times 30$

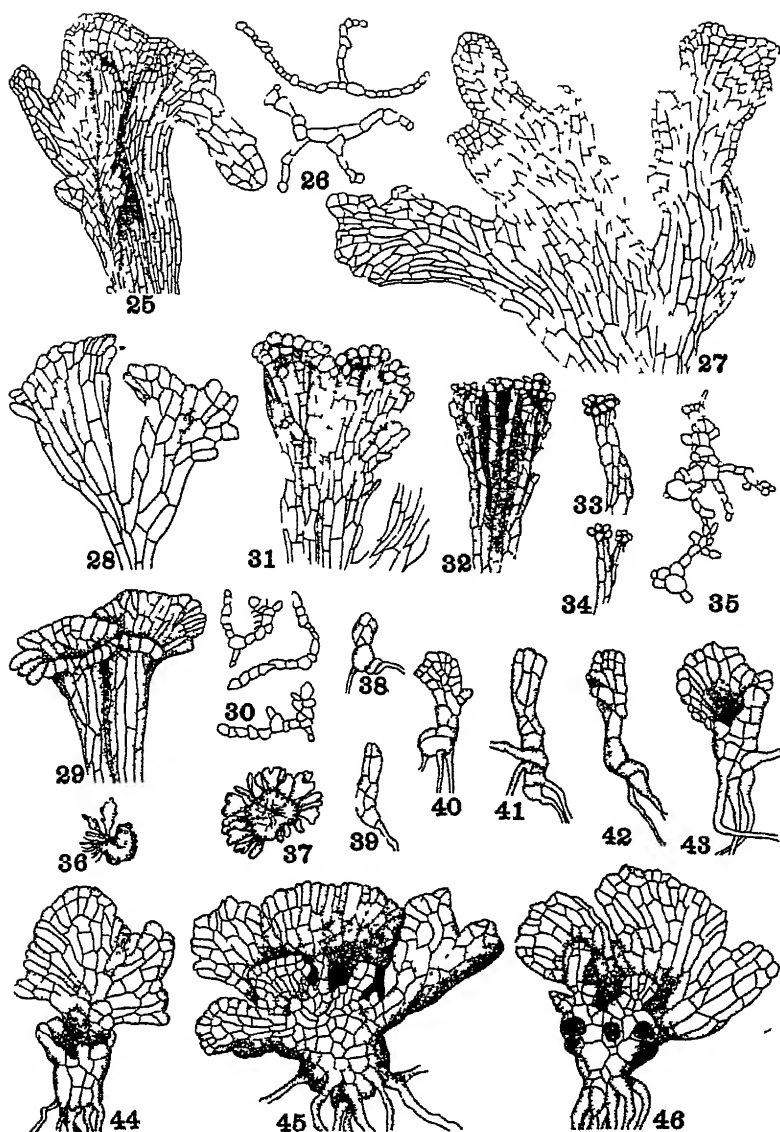
Not only is the first rhizoid long and positively heliotropic as noted by BUCHTEIN and CAMPBELL, but others of the early rhizoids turn in any direction. This often gives a culture one to three weeks old the appearance of being overrun by fungi. Many rhizoids, however, early penetrate the substratum and firmly anchor the thalli.

The persistence of the exospore following germination is variable. More commonly it is not evident after germination (figs. 2-6), although the elaters often remain about the first rhizoid (fig. 4). In some cases, however, the exospore remains until the thallus is of considerable size (fig. 7).

Soon after differentiation of the two body regions, the upright green branches assume the form characteristic of the species. In *E. kansanum* these consist of a column of one to several cells in thickness, at whose top are many small lobes consisting of one or a few cells each (figs. 7-9, 31-34). The tips of these branches, massed closely together, give the surface of the gametophyte a compact, almost granular appearance. Cross-sections of these branches are shown in fig. 35.

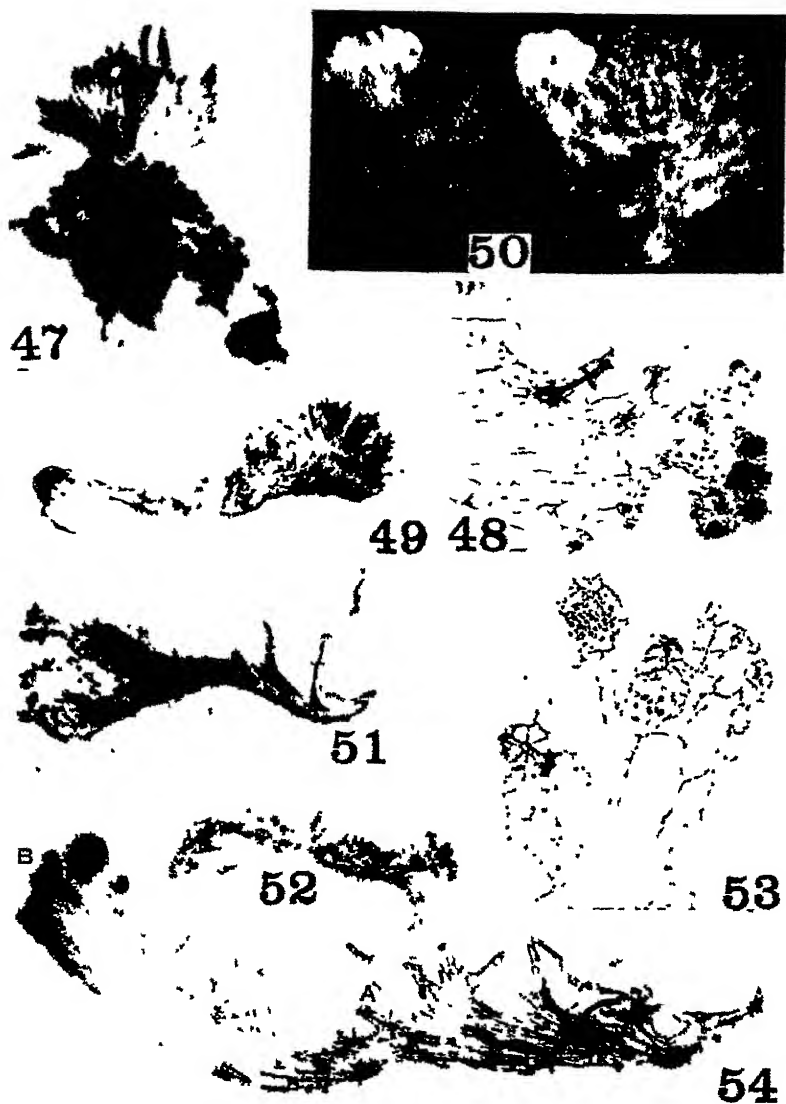
From the time the massive base and region of meristem are differentiated, further growth is variable and apparently dependent upon external conditions. If the planting is thick, so that the thalli are much crowded at once, very little chlorophyll bearing tissue is formed, and few, sometimes no, upright branches appear. Such thalli form antheridia when about 20-30 days old, and soon die. These are the so-called male gametophytes. Normal uncrowded plants, however, or the more vigorous of the crowded ones, form many upright green branches and masses of rhizoids. These (figs. 8, 9) grow 30-40 days before formation of either archegonia or antheridia takes place. The two body regions are well established (figs. 8, 9) before the sex organs begin to be differentiated. On these normal prothallia it is more common for archegonia to appear first, although antheridia first form on some such thalli. Those forming archegonia look in every respect like those forming antheridia, until the sex organs develop. The archegonia, as described by the writer (9) for *E. laevigatum*, occur on the massive tissue between the upright green branches. They are formed in the meristematic rim of the thallus at the same time that the branches are formed, and when mature come to lie between two of these branches. Continued growth of the meristem leaves the archegonia with their accompanying branches distributed over the upper surface of the thallus.

If the first archegonium is fertilized, the growing embryo soon exhausts the young thallus and it does not develop further. Thalli, such as those shown in figs. 8 and 9, normally contain one or more archegonia. If, however, at this stage antheridia develop instead of archegonia, they are formed in the region of meristem (fig. 8a), after it has considerably expanded and has ceased to develop green branches. In such cases usually the entire region of meristem becomes a mass of antheridia, and further development does not occur.



FIGS. 25-46—Figs. 25-35, upright green branches of three species of *Equisetum*; figs. 25, 27, branches of *E. telmateia*; fig. 26, cross-section of same; figs. 28, 29, branches of *E. arvense*; fig. 30, cross-sections of same, figs. 31-34, branches of *E. kansanum*; fig. 35, cross-sections of same; $\times 30$ Figs. 36-46, *E. telmateia*: fig. 36, gametophyte with one archegonium and many antheridia, $\times 5$; fig. 37, thallus from ventral side showing massive base bearing archegonia about periphery, $\times 5$; figs. 38-46, early stages in development of gametophyte of *E. telmateia* (figs. 44 and 45 had archegonia beneath upright lobes; fig. 46 as figs. 44 and 45 and antheridia); $\times 30$.

If some meristem persists after the maturing of the antheridia and a rest period, it may renew its growth and later develop either archegonia (fig. 49) in a mass of upright green branches, or more antheridia after a period of growth. In the plant shown in fig. 49, the base (left in figure) of the thallus is so decomposed that its sex cannot be determined definitely, but a few remnants of upright branches indicate that it had been archegonial. To the right of this darkened base is a typical antheridial region with no branches, few rhizoids, and many old and darkened antheridia. The right half is a continuation of the main axis, and is producing archegonia between the numerous green branches. Below these is the mass of rhizoids characteristic of archegonial regions. The most common condition is for the thallus first to form archegonia among upright green branches, and to continue to do so for some time if fertilization does not occur. After the formation of an indefinite number of archegonia, anywhere from one to a dozen or even more, the meristem region rapidly enlarges and becomes an expanded fan-shaped or club-shaped expansion devoid of chlorophyll and upright branches, and with few if any rhizoids. This expansion bears great numbers of antheridia. Fig. 50 shows two such thalli. Antheridia develop thus through a period of two to four weeks, when growth ceases and much of the thallus dies and becomes dark brown in color. It remains in this condition during a period of two weeks to two months, in which time many individuals die. Those that retain some living tissue then begin to grow, such growths starting from one cell or a group of cells in any part of the thallus. A thallus that has produced archegonia between the green lobes may spread out into many lobes bearing hundreds of antheridia. These lobes and the entire thallus may then become dark brown and appear dead. After as long as two months of dormancy, small groups of cells in the midst of the antheridial mass may begin to grow and develop into green thalli. The new growths in some cases were archegonial and had the characteristic mass of green branches and numerous rhizoids. However, in other individuals such new growths were antheridial. The tendency is for new active growths of this sort to be predominantly archegonial. Had these thalli been grown on soil it would have been impossible to have followed this development, for the older parts were so nearly decomposed that it was only after



FIGS. 47-54—Figs. 47, 48, 52, gametophytes of *E. telmateia*: fig. 47, thallus with archegonia between green branches and many antheridia, $\times 13$, fig. 48, section of growing tip of thallus with old archegonia and active antheridia, $\times 38$, fig. 52, dwarfed thallus with antheridia, $\times 10$. Figs. 49, 50, gametophytes of *E. kansanum*: fig. 49, thallus that has formed antheridia and is forming archegonia; fig. 50, two gametophytes that have many archegonia between their green branches and numerous antheridia on expanded tip of main axis, $\times 10$. Figs. 51, 53, 54, gametophytes of *E. onense*: fig. 51, thallus with many old archegonia between branches and many antheridia at tips of main axis, $\times 10$; fig. 53, section of wild thallus showing old archegonia, left and right, with antheridium between them, $\times 85$; fig. 54, elongated thallus with archegonium (a) and mass of antheridia (b); $\times 25$.

many attempts that a few such were successfully removed from the sphagnum. Many, however, were observed in place. By the time the new growth had matured and in turn formed antheridia, the original archegonial and antheridial regions had usually completely disorganized, and these new shoots appeared as did the original thalli (fig. 50). So large a number of thalli were observed to undergo these reversals in sex (figs. 49, 50) that they are believed to be normal. The small, so-called "male gametophytes" are only abnormal, crowded, and consequently starved thalli which produce a few antheridia and die. If by the death of surrounding thalli, however, a few of these dwarfed "male" plants secure room to grow while they still contain active cells, they develop into characteristic archegonial plants and function as normal thalli.

This alternation in the production of archegonia and antheridia was observed in many individuals in one culture over a period of two years, and for shorter periods in many cultures. During January and February of the first year, and during December and January of the second year, the cultures remained dormant. In each case after this midwinter period of dormancy there followed a period in which only archegonia were being formed by all gametophytes. No living antheridia were present during these periods. At all other times the cultures were predominantly either archegonial or antheridial. During such periods of archegonial dominance some gametophytes formed antheridia, however, and during periods of antheridial dominance some thalli were forming archegonia. Usually only one kind of sex organ was being formed on a thallus at a time. At the time the reversal was occurring, however, especially in the change from female to male, active archegonia and antheridia often occurred close together on the same thallus. In the reverse change this was not observed, for in all cases following antheridial development there was a period of dormancy, followed by one of growth before archegonia were again formed. There were many cases, however, where a thallus at the same time had several lobes, some of which were archegonial and others antheridial.

No regularity was found in this reversal of sex. In general the dominance of archegonia and antheridia changed about every 20-30 days; however, longer and shorter periods occurred. Still further

there was no set sequence. While more commonly a period of archegonial development was followed by one of antheridial development, gametophytes occurred that rested after archegonial development and again produced archegonia. This often occurred repeatedly and then antheridia sometimes developed. In a large number of the gametophytes death followed development of antheridia. Apparently every living cell was used in antheridial development. Death was rare following the period of archegonial development, but, as mentioned before, new thalli often arise from thalli that are apparently dead and from so little as one cell of such a thallus. In some cases gametophytes were found on whose main axes there had developed archegonia, then antheridia, then archegonia and again antheridia. The older parts were so badly decomposed, however, that it was almost impossible to remove them from the substratum without mutilation. Satisfactory photographs of the few removed were impossible because the older parts were so darkened that they photographed as a black mass. Every imaginable combination and arrangement of archegonia and antheridia occurred. A thallus might fork, one or both lobes forming archegonia; from one of these two lobes might arise, one antheridial and the other archegonial. The other of the two original lobes form either archegonia or antheridia in any sequence. Lobes may arise at any point on either male or female areas, and produce antheridia or archegonia without regard to the sex of the region from which they sprang.

The thalli grew thus, lobing and lobing again in all directions and producing archegonia and antheridia for the entire two years, although the number of individuals was greatly reduced owing to the death of many following the development of antheridia. While many branches arise from gametophytes and become separated by the decomposition of the older parts, this multiplication does not equal the mortality following periods of active development of antheridia. Also multiplication ceases when sporophytes develop. It is only in the case of very large gametophytes like those previously described by the writer (9, pl. XXIII, figs. 9, 17) that growth continues after sporophytes begin to develop from them.

Because the cultures were rarely watered, fertilization depended largely upon water condensed on and dropping from the cover of

the dish. This gave occasional sporophytes throughout the period, but following watering they appeared in large numbers.

The thallus starts as a narrow elongated structure with meristem at its tip; it dies in the older parts and decomposes as the meristem region widens, lobing more or less as the case may be. Thus the mature thallus comes to have in general a reniform shape, and is surrounded by a border of meristem. The notch represents the location of the older parts of the thallus.

Various attempts were made to find an explanation for the reversal of sex. It was thought at first that water might be the determining factor. It was found, however, that if of two cultures, both producing archegonia (or antheridia), one was watered and the other not, they would both produce the opposite sex at nearly the same time. With a number of cultures, some watered and others not, all would change approximately at the same time from a predominance of archegonia to one of antheridia and back to one of archegonia. Between periods of watering, cultures often reversed the dominant sex several times. It became evident that water was not the determining factor.

Light also could not have been the cause of reversal, as the cultures remained at all times in the same place and with the same side toward the light. Then too the reversal took place too frequently for length of day to be the cause. What brings about the change was not ascertained, but one fact is suggestive. Archegonia always develop at a time when upright green branches are forming and are active in photosynthesis. This is shown by the fact that they are between these branches and are formed at the time the branches are formed. Also large numbers of rhizoids are formed beneath the archegonial areas (figs. 49, 50). After such a period of active growth and photosynthesis, suddenly the peripheral meristem expands, ceases to form rhizoids, and branch rudiments fail to mature. From this tissue, nearly free of chlorophyll, many antheridia develop. Apparently they develop until the meristem is exhausted, or possibly until the chlorophyll-bearing branches of the archegonial region become old and cease to supply nourishment. Further development of sex organs then does not occur until active chlorophyll tissue is again developed, and this is usually associated with the develop-

ment of archegonia. If archegonia fail to develop, the green branches develop for about the same length of time and then antheridia are formed, as on gametophytes having produced archegonia.

Many sections were examined to determine whether reserve food occurred at one period and not at another. While many individuals contained much starch and others none, no definite relation could be found between this and the production of either antheridia or archegonia. At the time of climax production of antheridia, starch was scarce. It was commonly, although not always, abundant at the beginning of antheridial and during archegonial development. However, its presence at this time was not sufficiently uniform to be of much significance.

EQUISETUM ARVENSE

The gametophytes of *E. arvense* were found by the writer in but one place, near Weeping Water, Nebraska. These grew on moist clay soil situated in a small bend of a creek. The bank had a north-east exposure and was heavily overhung by trees on the south and west. The bank sloped abruptly from the creek and formed a horizontal surface 1-3 feet above the water level. The gametophytes occurred on the moist soil of this somewhat level surface. The area was covered with adult sporophytes and through it ran a little-used footpath. While the gametophytes were found throughout the area beneath the mature sporophytes, they were larger and more numerous along the path where the soil was packed. Here too was less competition with other plants. The gametophytes were found in the middle of June, and so were well advanced. Very few antheridia and archegonia were present. Most of the thalli bore well developed sporophytes, but some active archegonia and antheridia were present. Fig. 53 shows part of a section of one of these thalli. At the left is an old and darkened archegonium. At the right is an obliquely cut archegonium with a few celled sporophyte in its venter. Between the two is an active antheridium.

In this species, as in *E. kansanum*, development of the gametophyte was followed in plants grown on sphagnum. Here as in the other species a heliotropic rhizoid is formed from the basal cell, while the larger cell resulting from the first division continues to di-

vide in any plane. Subsequent divisions also occur in any plane and without order (figs. 10-23). In some cases (figs. 13, 17, 19) filaments consisting of several cells develop, while in others the formation of a massive tissue starts at once (figs. 15, 16, 21, 23). Also, as in *E. kansanum*, there is early differentiation of the two body regions (figs. 14, 20, 22), the massive base and the upright green branches. Here also badly crowded and dwarfed individuals do not develop normally, but form flattened masses of cells which produce many antheridia, the so-called male plants. These soon die. Thalli having sufficient room for normal growth, however, form the green branches and numerous rhizoids from the massive base on which sex organs soon appear. Some of the green branches of *E. arvense* are flat (fig. 28) and one cell thick, while others are two to three cells thick. Many of these broaden at the top into the form of a funnel (fig. 29).

Of the thalli that have room to grow normally, some may, after developing a few green branches, produce antheridia without having formed archegonia (fig. 24). These also usually live but a short time. If they live, they later produce archegonia.

By far the majority of normal thalli, however, first form archegonia. Anywhere from one to many archegonia develop. Then the meristem suddenly ceases to form green branches, expands, and usually turns upward (figs. 51, 54). Masses of antheridia are formed in these upturned extensions of the main axis. The thalli shown in figs. 51 and 54 had produced an archegonium at the base of nearly every green upright branch. The older parts of the thalli show the darkening characteristic of old tissue, while the upturned tips show the active antheridia. The antheridia of *E. arvense* protrude from the surface of the thallus more than do those of the other species (figs. 51, 54). Fig. 54 shows a long thin thallus of this sort, produced by growth toward the window. At *a* is an old archegonium. Younger ones, out of focus in the photograph, were present between other branches, while many antheridia show in the youngest tissue (*b*) at the extreme left.

Six weeks after planting, many of the flat crowded gametophytes had produced antheridia in large numbers and were dying. The less crowded larger gametophytes had the usual upright green branches. Most of these had developed no sex organs. A few bore only an-

theridia, while a few had produced one archegonium and were developing antheridia. All active thalli had upright green branches. When eleven weeks old practically all plants that formerly had borne antheridia were dead. The remaining plants had grown toward the light and had attained lengths up to 5 mm. and widths up to 3 mm. Nearly all bore many archegonia, and many were producing antheridia at the tips of the branched upturned axis (figs. 51, 54). Many others continued to form archegonia. After a period of rest, as in *E. kansanum*, new growths started from all surviving thalli. These were archegonial or antheridial without regard to the sex of the region from which they sprang; however, this renewing of growth took place to a much less extent than in *E. kansanum*. In most cases the gametophytes grew vigorously until one of two things happened. When fertilization took place and embryo development began, the gametophyte became exhausted and further growth ceased. If fertilization did not occur, development of archegonia continued for a greater or less time, but eventually antheridia formed at the tip of the axis. The meristem was all used in their formation and the thallus died. Very few such plants survived again to form archegonia. At the end of eight months all gametophytes had died, either from development of sporophytes or from the production of antheridia.

EQUISETUM TELMATEIA

The gametophytes of *E. telmateia*, like those of *E. arvense* but unlike those of *E. kansanum* and *E. laevigatum*, previously reported by the writer (9), have been found in close proximity to the plants from which the spores were discharged. Like the other species studied, these gametophytes were found only on clay soil; unlike the others they occurred only on vertical surfaces.

At Longbranch, Washington, they were found on a vertical bank near a waterfall, where there was an outcropping of clay. The bank sloped to the east and was heavily shaded by trees. Mature sporophytes overhung the bank. Near the Puget Sound Biological Station at Friday Harbor, Washington, gametophytes in all stages, from a few cells to 3 mm. in diameter (figs. 38-46), were found during the period from June 24 to July 25. These grew on the verti-

cal walls of a pit in which a water reservoir stood. The pit was in the northeast side of a hill. The growth of gametophytes was so dense as completely to cover the clay soil. They afforded ample specimens for study during this period. In this species all illustrations of developmental stages were made from these specimens found in nature. Mature sporophytes overhung the edges of the pit above the gametophytes. In the same vicinity gametophytes were found also, on the vertical sides of a small ditch in the midst of an area covered by sporophytes. Here the soil contained some sand. The walls of the ditch were deeply shaded by masses of *Equisetum* and ferns. On June 23 of the same year many gametophytes varying in size up to 3 mm. in diameter were found at Olga, Washington. These were on a steeply sloping bank on the shore of Puget Sound. However, they occurred only on vertical surfaces of clay outcroppings. The ground was kept moist by seepage from springs above. The exposure was east and shaded by trees. Many adult plants were found higher on the bank. During July and August large numbers of mature gametophytes, many of them bearing sporophytes 2-10 cm. high, were found near Forest Grove, Oregon. These were in a deep wide ditch running east and west and draining water from a hill covered with adult sporophytes. The ditch had been roughly dug. Its walls were of clay and vertical, and in its bed, which carried only a small amount of water, were many elevations extending 15-30 cm. above the water level. Gametophytes of varying size up to 7 mm. in diameter were thickly scattered over all moist vertical surfaces, but while the horizontal surfaces of the elevations in the bed were well moistened, no gametophytes were found on them.

The gametophytes of this species were thus found at five different places in great abundance. In all cases they were on only vertical surfaces of clay soil. All were in places where more or less shade occurred during the greater part of the day. In all locations there was a constant supply of moisture. Gametophytes grown on sphagnum agreed in all essential details with those found in the native habitat. Here they grew best on vertical surfaces, but they also did well on horizontal surfaces that were not unduly wet. It would seem that well drained moist surfaces and reduced light are essential for the growth of these prothallia.

On the germination of the spores, as is the case in the preceding species, the first divisions occur in any plane and are followed by other divisions without order (figs. 38-43). The first rhizoid arises from the lowest cell and is heliotropic. Other rhizoids arise from any of the early thallus cells and turn in any direction. As in *E. kansanum* and *E. arvense*, there is early differentiation of the two body regions, the massive base and the upright green branches. The base, as in other forms, soon develops a meristem which widens and often lobes until it forms a more or less circular body, the upper surface of which is covered with the upright green branches. Its under surface is thickly covered with rhizoids. The upright branches of this species are different from those of the other species studied. They are broad and usually one cell thick, except at points from which lateral platelike lobes arise (figs. 25-27, 43-47). They resemble somewhat the flat type of branches found in *E. arvense*. Because of the broad flat branches, early stages (figs. 44-46) might be mistaken for fern prothallia. At the stage shown in fig. 37, when seen from above, a gametophyte looks like a clump of fern prothallia standing more or less erect from the base. In older plants the basal axis may become much more lobed than is shown in fig. 37. In all plants taken from the native habitat, however, the circular form is roughly maintained. Plants grown in culture show more lobing of the base than do those growing in the open, but the lobes tend to radiate in such a way as to give eventually a circular appearance.

As in the preceding species, crowded cultures produce many slender, dwarfed, flat plants which give rise only to antheridia (fig. 52) and soon die (the so-called male gametophytes). The less crowded and more vigorous individuals form the massive base and upright green branches (figs. 44-46). In all cases examined, as soon as the first green branch is developed (figs. 43-46), an archegonium forms between it and the rudiment of the next green branch. It lies beneath the branch so that it is not readily seen. Often but one archegonium forms. If this is fertilized the gametophyte develops no further. If fertilization does not occur the meristem may at once enlarge and produce a mass of antheridia (figs. 46, 47). These are commonly known as "male thalli." It is not strange that the one archegonium between the green branches is easily overlooked.

Two months after planting, all normal gametophytes contained archegonia, and two weeks later sporophytes were visible. Most of these had developed from the first archegonium formed by the thallus. At an age of three months nearly all of the early crowded gametophytes had died, but many antheridia were forming on the meristem of thalli that had produced one or more archegonia. Other gametophytes continued to develop archegonia for some time before antheridia were produced. At four months some gametophytes had attained a diameter of 8 mm. and sporophytes 5-8 cm. high were present. The long, thin upright branches are loosely set and the large archegonia are conspicuous between them.

In a majority of plants more than one archegonium develops, and their formation may continue indefinitely, giving rise to the so-called female plants (figs. 37, 44-45). In all cases observed in culture, however, if fertilization does not occur, these archegonial thalli eventually develop antheridia from the meristem of the main axis or of some lateral branch of it. Fig. 48 shows a vertical section of such a thallus. Parts of two archegonia show, one darkened by age. The one nearer the antheridia still shows the egg, although the neck is darkening and shriveling. At the right is the antheridial region.

While this species did not grow as vigorously in culture as did *E. kansanum*, a limited number of thalli were observed over a period of two years. They passed through the same changes of sex as described for *E. kansanum*. As in that species, it is rare to find active archegonia and antheridia on a thallus at the same time, but repeated reversal of sex, both from female to male and back to female, were observed. Except for some early dwarfed individuals, all bore at least one archegonium before forming antheridia.

Only two cases were observed where antheridia were formed without the development of a more or less massive tissue. In one of these the thallus was but two cells thick; in the other case two antheridia were formed at the tip of an upright green branch.

Discussion

It is interesting to find that the gametophytes of the three species of *Equisetum* studied differ in the type of their habitat and in the form of their green branches. The branches are so characteristic

that the thalli can readily be distinguished by them; nevertheless their general mode of growth and reproduction are the same. All agree with *E. laevigatum*, previously described by the writer (9), and *E. debile*, described by KASHYAP (7), in being characteristically monoecious. The thalli that have usually been considered male plants are only the crowded and greatly dwarfed individuals. Those that have been considered female are the normal individuals which form both archegonia and antheridia. In all the species these normal plants characteristically produce first archegonia and then antheridia. Not only was this found to be the case in plants grown in culture, but also in those found in their native habitat. From these studies it becomes evident that the gametophytes of *E. laevigatum* previously described (9) were found just when the change from development of archegonia to that of antheridia was taking place, and hence the monoecious nature of the thalli was evident.

The occurrence of antheridia and archegonia on the same thallus was observed by BUCHTEIN (3), but the complete significance of his observations was not appreciated. Many who have worked with gametophytes of *Equisetum* have observed an occasional antheridium on a female plant, and vice versa. Probably the reason this has not been more commonly observed and its significance realized is that the same individuals have not been under observation for extended periods. Plants grown in culture just until archegonia begin to develop would give the impression of being dioecious. The mature ones taken from the native habitat at any one time might or might not show the two sexes. Only in large and vigorous thalli, such as those previously described (9), will growth and development of sex organs be continued after sporophytes are formed.

BUCHTEIN (3) observed that changes of light did not bring about changes in sex. He found, however, that crowded sowings produced few female plants and thin sowings produced many. He found that spores grown in pure water or on washed sand produced only male plants, while female plants removed from loam to sand produced only antheridia. He concluded that nutrition must in some way be the determining factor. BOWER (2) showed that even among homosporous ferns, whose thalli are recognized to be monoecious, gametophytes starved by crowding become reduced and bear only anther-

TABLE 5 also found in homosporous ferns that monoecious is normal, but that either sex may be suppressed so that the plant may become dioecious. He considers this due to external

It is evident that *Equisetum* agrees with the homosporous ferns in that its gametophytes, which are normally monoecious, may under crowded conditions of growth appear dioecious. As found commonly in nature and in cultures, the large gametophytes over-run many other thalli which are crowded beneath them and produce only antheridia. In cultures it was found that if the large thallus dies or is removed, some of these crowded thalli take on vigorous growth and begin to develop archegonia.

Summary

1. Gametophytes of *Equisetum kansanum*, *E. telmateia*, and *E. arvense* were found in large numbers in their native habitats, each of the three species having a characteristically different one.

2. All three species were grown to maturity on sphagnum, and such plants agree in all essential points with those found in their natural habitat.

3. The gametophytes of all species agree in their early development and at maturity in having a massive base, a meristematic rim, and upright green branches. The upright green branches are characteristically different in each of the species.

4. In all species archegonia and antheridia are borne on the massive tissue of the main axis or of its lobes. Only rarely do antheridia occur on the upright branches.

5. Normal thalli are monoecious. Dwarfed and starved plants produce only antheridia.

6. Archegonia develop from meristem that is forming upright green branches and many rhizoids, and at maturity lie between these branches.

7. Antheridia develop from meristem which is forming reduced branches or none, and few or no rhizoids. At maturity they occupy expanded chlorophyll-free regions. Following development of antheridia, the thallus rests for a time before again developing archegonia.

8. On normal thalli it is usual for archegonia to develop first, then antheridia; still later archegonia may again develop, followed by antheridia.

9. Development of antheridia brings about exhaustion of the thallus and results in the death of many plants. Fertilization and the consequent development of an embryo also bring about cessation of growth in the thallus. Exceptions occur only in case of very large thalli, which may continue growth with the formation of additional sex organs, until several sporophytes have developed.

10. In degenerating thalli any cell or group of cells may return to active growth and develop lobes which function in all respects as the original thallus.

11. Cultures of *E. kansanum* and *E. telmateia* have been under continuous observation for two years; those of *E. arvense* for eight months.

The writer is indebted to Professor J. H. SCHAFFNER for identification of the plants from which the spores were obtained for cultures, and to Professor T. J. FITZPATRICK for careful reading of manuscript and proof. For many courtesies the writer is also indebted to Dr. T. C. FRYE and the Puget Sound Biological Station, where much of the work on *Equisetum telmateia* was done, and to Dr. G. R. LARUE and the University of Michigan Biological Station, where the paper was completed.

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[Accepted for publication December 10, 1930]

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DEVELOPMENT OF THE MACROGAMETOPHYTE AND EMBRYO OF *DAUCUS CAROTA*

H. A. BORTHWICK

(WITH THIRTY-TWO FIGURES)

Introduction

The work reported in this paper, while dealing mainly with embryogeny of *Daucus carota* L., also includes some observations on the structure of the female gametophyte and on the development of the pollen tubes. The embryogeny of the Umbelliferae has not been investigated extensively, but the few species which have been examined seem to have certain features in common. In all of them the embryo develops into an unusually long structure before longitudinal divisions occur. The sequence in which the cells divide is extremely variable among individuals of the same species. SOUÈGES (17), who has made a careful study of *Carum carvi* L., finds that its embryonal development is not essentially different from that of certain members of the Solanaceae (15) and the Rubiaceae (16). Further critical studies of embryogeny of other members of the Umbelliferae, therefore, have seemed desirable for comparison with the few which have been studied.

The carrot (*Daucus carota*) was selected for this study because it was thought that such an investigation might throw light on practical problems connected with seed germination. These studies of *Daucus* show that a large percentage of the seeds fail to germinate. Examination of these seeds shows that the failure is due to defective embryos. While considerable information has been obtained during this investigation on the causes of the failure of embryos to develop, this phase of the problem will be discussed in a later paper.

Materials and methods

The carrots from which material was collected were of the Chantenay variety. Most of the plants were grown at Davis, California; others were grown by C. C. Morse and Co. near Clarksburg, California. The material was killed and fixed in a solution made as fol-

lows: solution A, 4 parts of commercial 40 per cent formalin and 1 part water; solution B, 1 gm. chromic acid and 10 cc. acetic acid in 90 cc. of water. Equal parts of the two solutions were mixed at the time they were used. Material showing stages in the development of the female gametophyte and the early embryogeny were stained with Haidenhain's iron-alum-haematoxylin. Delafield's haematoxylin was found very satisfactory for later stages in embryonal development. Pollen tubes were well differentiated with a combination of Haidenhain's haematoxylin and resorcin blue. The points in which this special technique for staining pollen tubes differs from that of BRADBURY (3) will be described in a later paragraph.

Literature review

A survey of the literature dealing with the morphology of *Daucus carota* shows that comparatively little has been done on that part of the life cycle included under the title of this paper. The most extensive investigation is that of HÅKANSSON (7), who describes the development of embryo sacs of a great number of genera of Umbelliferae, including *Daucus*. He reports that in *Daucus* a single archesporial cell gives rise to four macrospores in a rather limited nucellus. The embryo sac, which is at first small, enlarges rapidly and ruptures the nucellus transversely. One or more nucellar cells frequently persist next to the tip of the expanding embryo sac. The chalazal portion of the ruptured nucellus is conspicuous in the lower part of the ovule, and in it the antipodals remain evident long after fertilization. HÅKANSSON makes no reference to the details of fertilization, and merely states that the embryo has a long suspensor. MEZ (12), generalizing on the mature embryos of Umbelliferae, states that the calyptra of the root is always well developed and the plumule always absent. He says that the embryo of *Daucus* is elongate and has broadly linear cotyledons that are as long as the radicle.

Although the macrogametophytic development of *Daucus* has been briefly described by HÅKANSSON, other members of the family have been investigated more thoroughly in this respect. HÅKANSSON, in his general discussion of the family, states that archesporial cells function directly as macrospore mother cells without giving rise to any parietal tissue. He found, however, that in a number of

members of the family a multicellular archesporium is produced, a fact which BEGHTEL (2) also reported for *Pastinaca*. In nearly all cases examined HAKANSSON found that four macrospores are produced, and that the inner gives rise to an 8-nucleate embryo sac in the usual manner. This differs from BEGHTEL's account of *Pastinaca*, in which he says, "It appears that the outer megaspore invariably functions." He bases this statement on the fact that in the chalazal end of the nucellus there is a mass of dark staining, disintegrating tissue which he assumes to be the disintegrating spores. This interpretation is open to question. It seems more probable that this dark staining material is not disintegrating spores but is the tissue found in many umbelliferous plants which HAKANSSON regards as similar to the hypostase of the Onagraceae. Nothing particularly unusual has been reported concerning the macrogametophytic development in the family, aside from the early rupture of the nucellus and the rapid enlargement of the embryo sac at that time. HAKANSSON finds a more or less striated appearance in the micropylar part of the synergids, but states that a filiform apparatus is not present.

The details of embryo development are not well known for the family as a whole. HAKANSSON shows a few figures of early development in *Foeniculum vulgare*, *Carum carvi*, and *Anethum graveolens*, and records the fact that HEGELMAIER (8) has studied similar stages of *Petroselinum*, *Bunium bulbocastanum*, and *Eryngium bulbosum*. In all of these species it is apparent that the 4-celled embryo is linear. The next divisions are also transverse in many of them, so that filamentous embryos eight or more cells long, in which no longitudinal divisions have taken place, are common. In *Eryngium yuccifolium*, JURICA (9) reports the presence of a long suspensor and of considerable diversity in the sequence of cell division, but his figures are all of rather advanced embryos. TANTANI (19) says that embryos, 10-14 cells in length without longitudinal divisions, are not uncommon in the Apiaceae.

In all of the papers mentioned development of the embryo was not the main problem, and consequently was not investigated at all thoroughly. SOUÈGES (17), working with *Carum carvi*, has recently published the most detailed embryological study yet made of the Umbelliferae. In this species he finds that a linear file of four cells

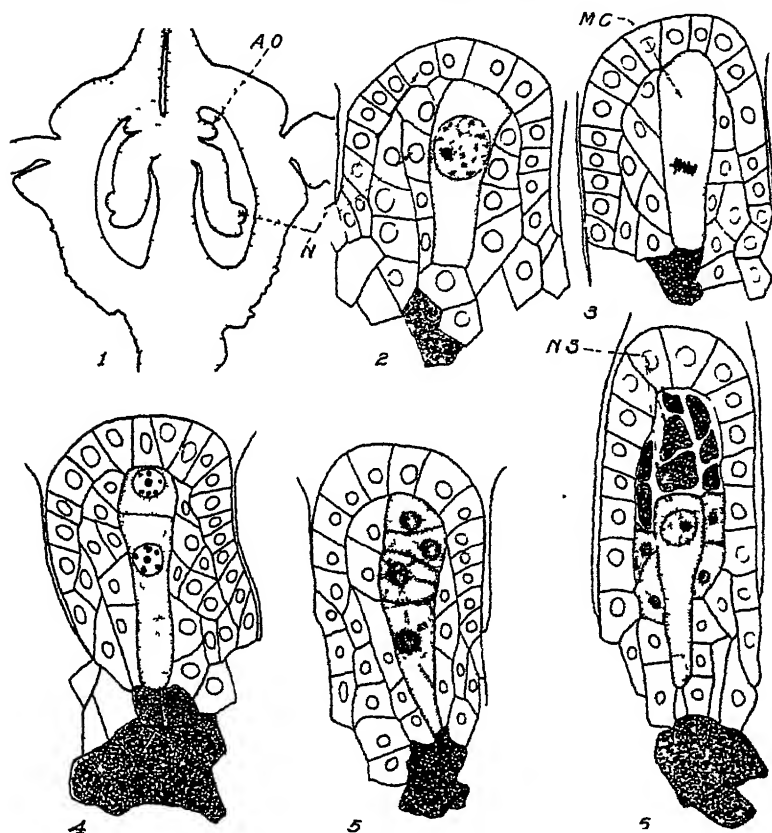
is invariably formed, after which the planes of division may vary. The two cells nearest to the micropyle almost always divide transversely while the other two behave in one of three ways. Both may divide longitudinally, the terminal one of the two may divide transversely and the other longitudinally, or both may divide transversely. The resulting 8-celled embryos are respectively six, seven, and eight cells long. Each of these 8-celled types is figured by SOUÈGES (17), and he shows a number of later stages in the development of each type into a mature embryo. Although these three types of 8-celled embryos are different, the mature embryos produced from them are essentially alike. In summarizing his work, SOUÈGES points out that there is but one type of 4-celled embryo which gives rise to these three different types of 8-celled embryos. He finds, moreover, that each cell in the 4-celled stage gives rise to certain definite parts of the mature embryo, regardless of variation in the sequence or planes of division. He concludes that in mature embryos of *Carum carvi* the cotyledons arise from the distal cell of the 4-celled embryo; the hypocotyl from the cell immediately below it; the root cap primordium and a portion of the suspensor from the next lower cell; and the remainder of the suspensor from the proximal cell of the four. SOUÈGES has made similar studies of representatives of a great many families, and his generalizations based on these studies will be discussed later.

Macrogametophytic development

At the time the archesporial cell first becomes evident in the nucellus, the ovule is pendent in the locule and the single integument has just begun to appear (fig. 1). Its nucellus consists of a small cylindrical mass of tissue which, when viewed in cross-section, varies from five to seven cells in diameter. At its chalazal end there can usually be found a number of dark-staining cells which appear to be in a state of disintegration. These become even more conspicuous a little later. This tissue is the hypostase to which HÅKANSSON refers and which BEGHTEL apparently confuses with disintegrating sporogenous tissue in *Pastinaca*.

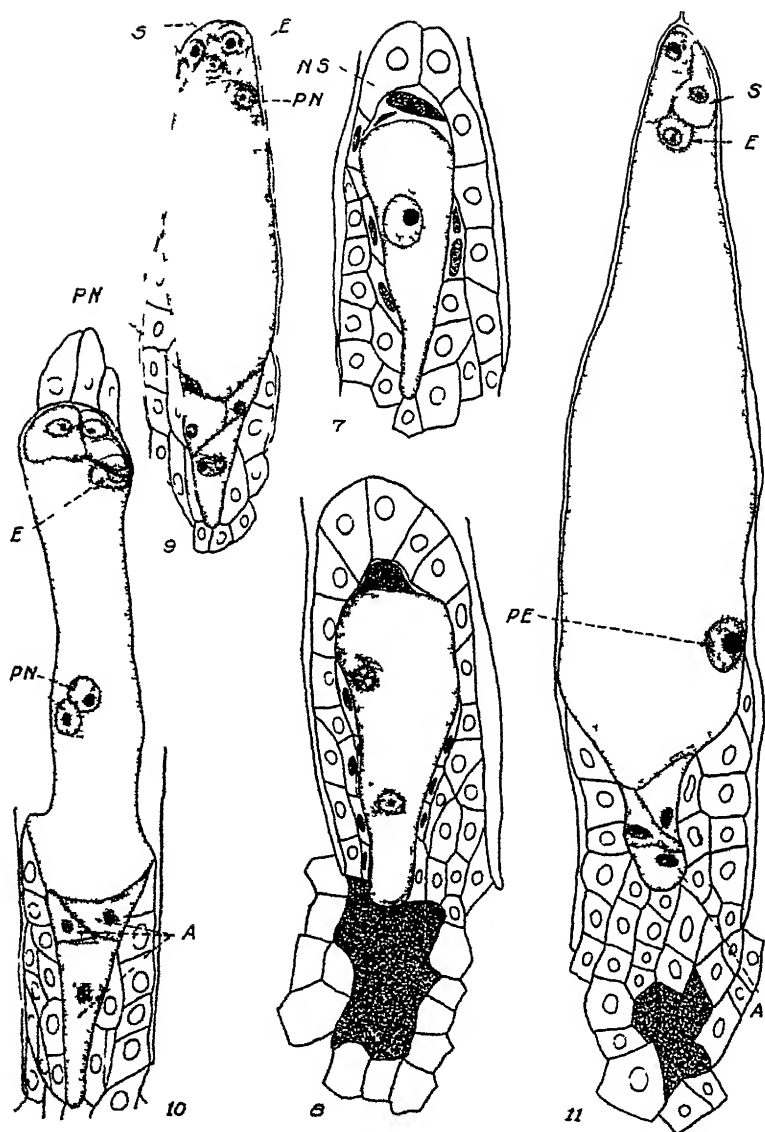
The single archesporial cell functions directly as a macrospore mother cell, a feature reported by HÅKANSSON as characteristic of

the family. Nothing unusual was found in the development of the macrospores (figs. 2-5). The mother cell gives rise to a linear tetrad with cytokinesis following each mitosis. The embryo sac arises from the innermost macrospore, which enlarges rapidly at the expense of



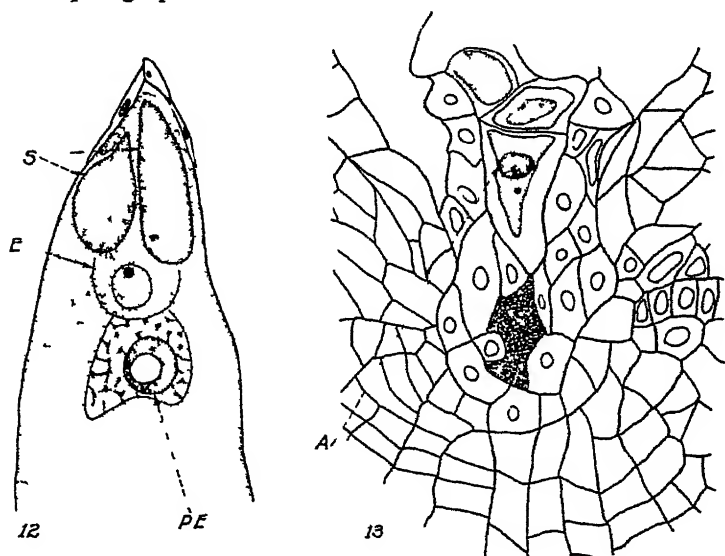
FIGS 1-6.—Fig. 1, longitudinal section of flower at time of macrospore mother cell formation. AO, abortive ovule. N, nucellus; $\times 80$. Figs 2-6, stages in macrospore formation. MC, macrospore mother cell; NS, non-functional spores; $\times 750$.

the other three (figs. 6, 7). The 8-nucleate condition is attained while the embryo sac is still only a fraction of its final size. As the macrogametophyte approaches maturity, the two polar nuclei migrate from opposite ends to the center of the embryo sac and fuse with each other (figs. 8-11). Fusion takes place well before fertiliza-



FIGS 7-11 —Successive stages in embryo sac formation. S, synergids, E egg, PN polar nuclei, NS non functional spores, A, antipodals, PE, primary endosperm nucleus, X-50

tion The primary endosperm nucleus thus formed then migrates to a position immediately under the egg apparatus where it remains until the time of fertilization (fig 12) It is large and contains an enormous nucleolus The egg at this time is somewhat elongate vacuolate in the upper part but with rather dense cytoplasm and a small nucleus at the lower end The synergids contain abundant cytoplasm with small vacuoles in their lower ends and filiform structure at their apices This last feature will be discussed more fully in another paragraph



FIGS 12 13—Micropylar and antipodal ends of embryo sac ready for fertilization
S synergids, PE primary endo-sperm nucleus 1 antipodal-, $\times 730$

During enlargement of the embryo sac the nucellar cells lateral to the non-functional macrosperes disintegrate (figs 6, 7) The nucellar epidermis remains intact, however, until it is finally ruptured transversely by the enlarging embryo sac (figs 9, 10) This usually occurs at about the time the sac reaches the 8-nucleate stage The nucellar cells which are thus torn loose from the chalazal part of the nucellus may be seen for a time at the tip of the egg apparatus (fig 10), but they soon disintegrate The chalazal portion of the nucellus, however, persists much longer In it the three antipodals may

be recognized until after fertilization (fig. 130). They usually have a triangular arrangement, with one somewhat elongate directed toward the chalaza and the other two side by side above it. After the nucellus is ruptured the embryo sac lies in direct contact with the inner epidermis of the integument. The ovule during these changes enlarges rapidly and the embryo sac keeps pace with this enlargement.

Although HANSSON states that a filiform apparatus of cellulose is not found in the Umbelliferae, I find evidence to the contrary in *Daucus*. The staining reactions of the somewhat striated, caplike apices of the synergids in the mature macrogametophyte give a distinct cellulose reaction. With safranin and light green, for example, the apices of the synergids stain green with small spots of red scattered throughout. These red spots become progressively larger and more abundant back from the apex. The green-staining continuous phase I interpret as being composed of cellulose, while the red-staining discontinuous portion is the cytoplasm inclosed in the minute cellulose chambers. With Haidenhain's haematoxylin and a light counter-stain of orange G, the tips of the synergids stain orange with small dark spots in them which have taken the haematoxylin. In this case the haematoxylin has stained the cytoplasm and the orange G the walls. Both of these staining reactions indicate the presence of cellulose in the tips of the synergids, but in order to prove the point more definitely microtome sections were treated with chloriodide of zinc according to ARTSCHWAGER'S (1) method. Synergids treated in this way show a definite blue color in that portion which takes the light green or the orange G. This shows that a definite filiform apparatus of cellulose is produced in *Daucus*. Its structure appears to be similar to that described by HABERMANN (6), but it is not developed as conspicuously as in the plants which he studied.

Pollen tube development

Preparations stained in the usual manner showed pollen tubes in such great abundance along the funiculus of the ovule and about the micropyle that it was thought desirable to devote special attention to their development. While the tubes could easily be traced where they were free in the locule, such was not the case in other

parts of the pistil. After a number of trials of various stains, resorcin blue, which was first used by TSVETT (20), was selected as the most satisfactory for tracing the path of the tubes through the tissues of the style. Paraffin sections, $10\ \mu$ thick, were stained in Haidenhain's iron-alum haematoxylin and counter-stained in a 0.5 per cent solution of resorcin blue for 1-24 hours. They were then washed quickly in water and mounted in Apathy's medium directly from the water. Mounting in this medium, made up as suggested by LEE (10), is much more satisfactory than mounting in balsam, since most of the resorcin blue is removed from the sections when they are passed through the grades of alcohol and xylol into balsam. These preparations dry quickly, in fact even quicker than those mounted in balsam. Slides prepared in this way have been kept for several months with no evidence of deterioration except for a slight amount of crystallization of the mounting medium around the edges of the cover slip, an effect that could probably have been prevented by sealing the mounts with gold size or some other cement. The only tissue in the preparations which stains blue with this method, other than the callose portions of the pollen tubes, is the degenerating part of the two abortive ovules which are situated almost directly above the point of attachment of the two fertile ones (fig. 14 *h*). Resorcin blue was found to be a somewhat satisfactory stain when used alone, since it stains the protoplasts of cells a reddish brown and the walls of the tubes a bright blue. Better definition is obtained, however, by using it as a counter-stain after the haematoxylin. Other stains, such as aniline blue and rosolic acid, were used but were found less satisfactory than resorcin blue.

At the time of pollination the pistil has the structure shown in fig. 14. The two cylindrical styles are 2-3 mm. long and diverge slightly from each other. The lower portions of the styles are grooved on their inner faces, a feature best seen in cross-sections cut at this level (fig. 14 *a*, *b*). Lower down the two grooves form a single slot-like canal in the fused bases of the styles (fig. 14 *c*) that communicates laterally with the locule of each carpel. There are three vascular bundles extending about two-thirds of the length of each style, one opposite the groove and the other two lateral to it. Between these three bundles and the grooved side of the style is a con-

ductive tissue composed of elongated cells. The upper end of the conductive tissue is the stigmatic surface. In each locule there is an

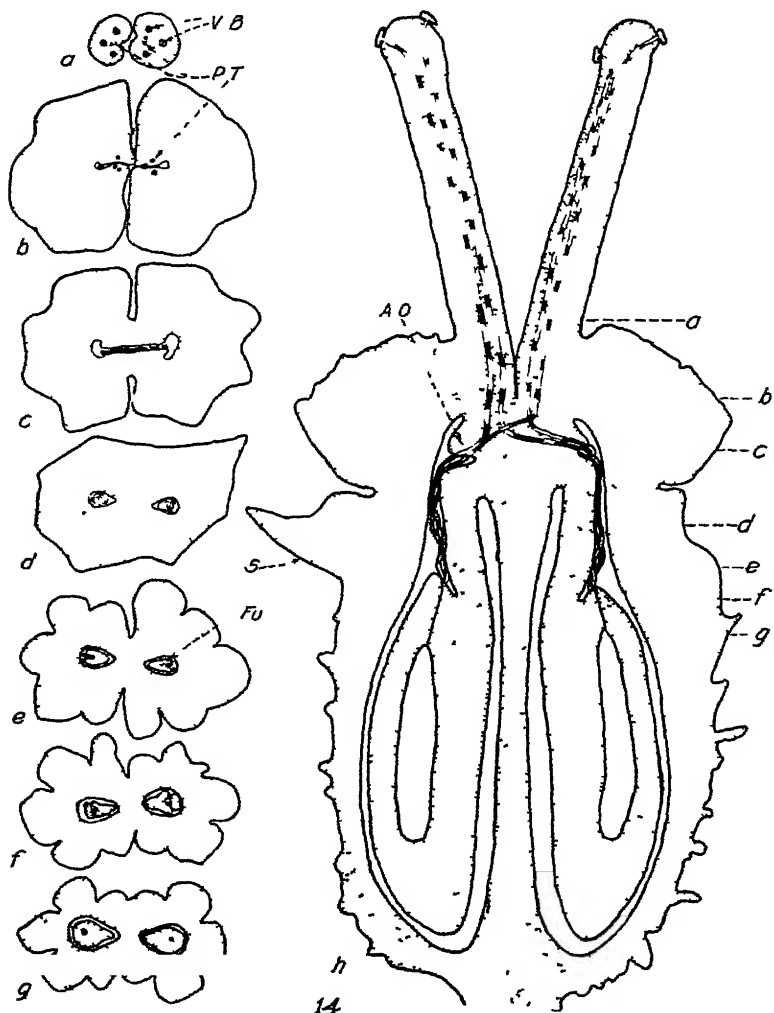


FIG. 14.—(1) cross and longitudinal sections of flower showing course of pollen tubes diagrammatically. *a*, cross sections taken at levels *a*–*g* of *h*, *VB* vascular bundles, *PT*, pollen tube, *Fu*, funiculus, *S*, sepal, *AO*, abortive ovule, *a*–*g* $\times 165$, *h*, $\times 30$

abortive ovule, and below it a larger functional one with a long funiculus and a single integument. Near its upper end the funiculus is

approximately cylindrical in cross-section (fig. 14 *d*), but lower down it has a groove which lies almost directly above the micropyle (fig. 14 *e, f*). Funicular cells along this groove have dense protoplasts and appear to be secretory in nature.

The pollen grains, germinating on the stigma send out tubes that grow between the cells of the stigmatic surface and intercellularly down through the conducting tissue (fig. 14 *a*). Toward the basal part of the styles the path of the tubes lies close to the stylar groove (fig. 14 *b*), and at a slightly lower level the tubes emerge from the interior of the style and grow along its grooved surface. At the level of the transverse canal (fig. 14 *c*) they may either cross over from one carpel to the other or continue downward in the same carpel. Both possibilities in the growth of pollen tubes have been observed repeatedly.

When the tubes have reached the locule they grow around or over the abortive ovule and to the point of attachment of the fertile one. From here their course is downward along the grooved surface of the funiculus to the micropyle. CAMMERLOHER (4), studying the ovules of a number of Umbelliferae, including *Daucus*, also observed this secretory funicular tissue along which the tubes grow, and states that it serves to conduct the pollen tubes to the micropyle. The tubes usually bend away from the funiculus as they approach the ovule, and pass directly into the micropyle and down it to the apex of the embryo sac. Sometimes, however, they follow down along the funiculus to the point where it joins the ovule and then up the surface of the integument to the micropyle. In two or three cases tubes were seen to branch as they entered the micropyle. Although this is not common in angiosperms, DAHLGREN (5) mentions a number of cases where it has been noted. While several tubes have been observed entering one micropyle, it is not uncommon to find others which have failed to reach the micropyle, growing for a short distance along the outer surface of the integument.

After entering the embryo sac the pollen tubes grow between the two synergids. The filiform apparatus is ordinarily left intact and can be identified for some time after fertilization. The cytoplasmic portion of the synergid, on the other hand, disintegrates soon after the entrance of the pollen tube. It is not unusual to find pollen tubes

growing past the egg apparatus. In one case a tube was traced for nearly one-third the length of the embryo sac. In this particular ovule fertilization had already occurred, and the endosperm consisted of about sixteen free nuclei distributed about the periphery of the embryo sac. The tube did not penetrate the cytoplasm of the sac but grew between it and the inner surface of the integument.

Although STRASBURGER (18) observed the occurrence of plugs in pollen tubes, the callose nature of these plugs and of the inner lining of the pollen tube wall was first thoroughly discussed by MANGIN (11). The distribution of the callose in pollen tubes of *Daucus* is not the same throughout their entire length. Near the stigma and in the upper half of the style, the walls of the tubes may stain a faint blue only, or they may take up sufficient stain to stand out sharply. The plugs, on the other hand, are conspicuous and give a strong callose reaction. They differ greatly in size and shape, and vary all the way from globular masses adhering to one side of the wall to transverse septa. They are usually 3-10 μ in length and are spaced at intervals of about 25 μ .

Toward the base of the style the plugs are longer and lie closer together than in the upper part. Lateral walls between plugs also show a callose reaction more frequently than at higher levels in the style. Portions of the tube within the locule and micropyle have fewer plugs, but the entire wall of the tube is frequently much thickened with callose. In some cases the lumen is reduced to one-third or one-fourth the diameter of the tube, and here and there it appears to be almost completely closed.

In older material, in which the embryo was 2-celled and the endosperm about two layers of cells thick, the tubes along the funiculus were found to have lost their capacity to stain with resorcin blue, although they could still be recognized in the unstained condition. In the micropylar region the tubes still stained blue but appeared somewhat disorganized. In the stylar tissue, however, the plugs appeared to stain even more conspicuously than at the time of fertilization.

Embryogeny

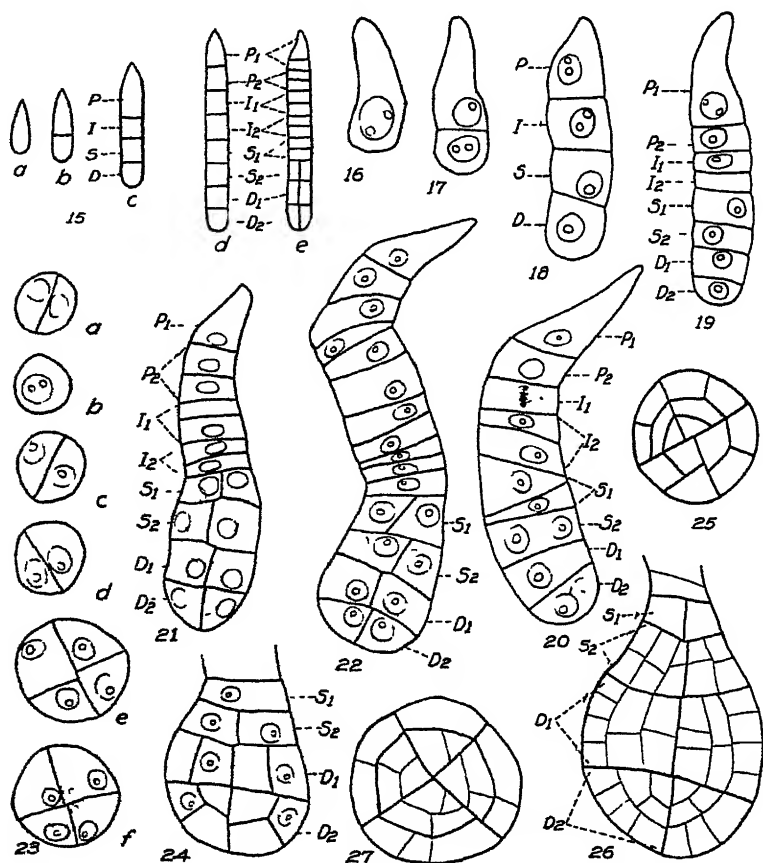
Although fertilization was not observed, the stages subsequent to it were found in abundance. Division of the primary endosperm nu-

cleus takes place soon after fertilization, and the number of nuclei becomes considerable before the zygote divides. These nuclei are numerous in the upper part of the embryo sac around the zygote; elsewhere they are confined to the periphery of the sac. The first few divisions of endosperm nuclei are nearly simultaneous, but toward the end of the free nucleate stage their order becomes irregular. The multinucleate endosperm becomes cellular about the time the embryo is 2-celled. After this the number of endosperm cells increases rapidly by cell division. The central cavity within the immature seed is soon nearly filled with endosperm tissue, while the embryo still contains only a few cells. Part of this tissue is digested as the embryo elongates and pushes into the central part of the seed. At maturity the seed is entirely filled with rather thick-walled endosperm cells, except for a small cylindrical region occupied by the embryo.

Development of the embryo begins with an elongation of the zygote (fig. 16). The first division is transverse. The daughter cell next to the micropyle is elongate, the other is small and rounded (fig. 17). Each of these cells divides transversely, giving rise to four cells that are always arranged in a linear series in *Daucus* (fig. 18).

The arrangement of cells in the 4-celled embryo and the way in which each of these takes part in the formation of the mature embryo are matters which SOUÈGES (13, 14) regards as of great importance in the embryogeny of angiosperms. He shows, as a result of studies of representatives of many families, that 4-celled embryos may be separated into two main types on the basis of the cellular arrangement. One type has the linear arrangement just described for *Daucus*, and is characteristic of such families as the Solanaceae and Rubiaceae. The other type has the two distal cells of the four side by side, making the embryo three cells long. This type, which is the more common of the two, is found in the Compositae, Ranunculaceae, Liliaceae, Cruciferae, and other families. In nearly all cases, all members of a family produce either one or the other of these 4-celled types. SOUÈGES has also carefully determined, for species both in the same and in different families, the exact parts of the mature embryo that arise from each cell of the 4-celled embryo. From these observations (13, 14) he draws two conclusions: (1) each cell of a

4-celled embryo gives rise to a definite region in the mature embryo which is frequently the same in all members of the family or larger group, but differs from one group to another; (2) the way in which



FIGS. 15-27. *—Fig. 15, a-e, diagrams showing usual course of development of *D. n.* embryo to 16-celled stage. Figs. 16-19, longitudinal sections of 1-, 2-, 4-, and 8-celled embryos. Figs. 20-22, longitudinal sections of embryos in about 16-celled stage and older. Fig. 23, a-f, successive cross-sections of embryo of approximately same stage of development as that of fig. 21. Fig. 24, longitudinal section of embryo somewhat older than that of fig. 22; suspensor not shown. Fig. 25, transverse section through embryo in about same stage of development as that of fig. 24. Fig. 26, longitudinal section of embryo with dermatogen periblem, and plerome initials differentiated; suspensor not shown. Fig. 27, cross-section of embryo in approximately same stage of development as that of fig. 26, $\times 750$.

* See page 37 for explanation of symbols used in figs. 15-27.

the derivatives of each cell of the four enter into the formation of the parts of the mature embryo constitutes a character which is of use in the determination of plant relationships.

Since it will be necessary to refer repeatedly to specific cells of the 4-celled embryo and their derivatives, they will be designated as follows: The cell next to the micropyle will be termed the *proximal* cell (P); the cell next below it will be termed the *infra-median* cell (I); the cell next below this will be referred to as the *supra-median* cell (S); while the cell farthest from the micropyle will be called the *distal* cell (D) (figs. 15 c, 18). In *Daucus* all cells of the 4-celled embryo nearly always divide transversely. The cells of the filamentous 8-celled embryo thus formed are designated throughout this paper in the following manner. The two derivatives of the proximal cell are referred to as P₁ and P₂, those of the infra-median cell as I₁ and I₂, those of the supra-median cell as S₁ and S₂, and those of the distal cell as D₁ and D₂ (figs. 15 d, 19). In all cases the subscript 1 refers to the proximal of two sister cells and the subscript 2 to the distal of the two. The few 8-celled embryos which arise in a manner different from that just described will be discussed later.

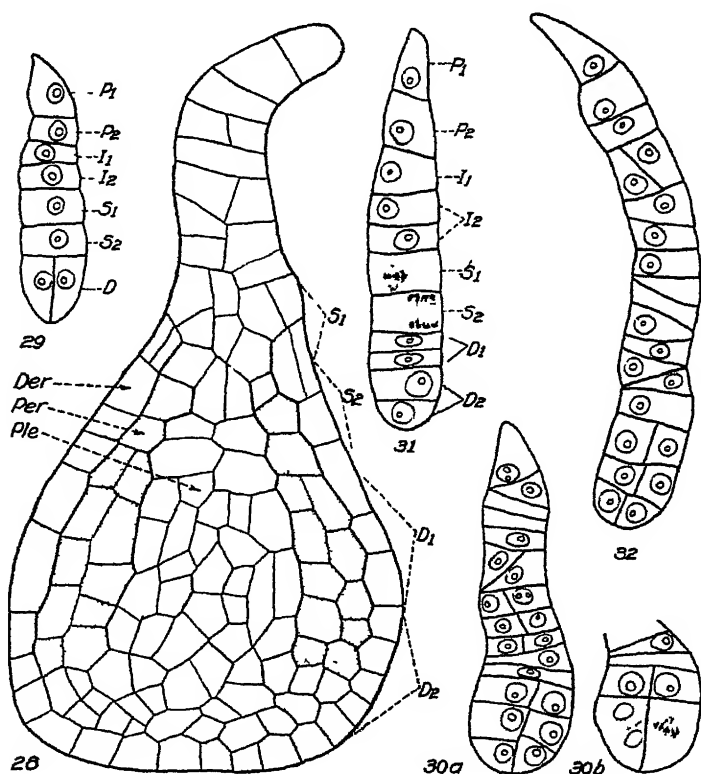
From the 8-celled stage onward the planes of division are less regular in orientation, and consequently more than one method of development may occur. The following account, based on the study of a great number of embryos, describes what is considered the most frequent course of development after the 8-celled stage. The three cells farthest from the micropyle (D₂, D₁, S₂) divide longitudinally; the other cells usually divide transversely. If these divisions were always in the planes just described, the result would always be a 16-celled embryo, 13 cells long (fig. 15 e). Such is seldom the case, however, since the plane of division in any of the five cells nearest the micropyle may be vertical or diagonal (fig. 20). In such cases the length of the embryo is correspondingly shorter. Moreover, the three cells farthest from the micropyle (D₂, D₁, S₂) may redivide before all of the other five have completed their first division, in which event the 16-celled stage of fig. 15 e never occurs. For example, in fig. 20 one of the cells usually dividing transversely (S₁) has divided diagonally, and another (I₁) is dividing vertically. Another example is shown in fig. 21, where the fifth cell (S₁) from

the micropyle has divided vertically. It will be noted that in these embryos there is a vertical division in the three distal cells of the 8-celled stage. The differences that arise are caused by the irregularity of direction or sequence of division in the five cells nearest the micropyle.

Before considering the various other types of development found in young embryos of *Daucus*, it may be well to follow the development of this first type through to maturity of the embryo. Interpreting the later stages on the basis of the 8-celled embryo, we find that the derivatives of the three distal cells (S_2 , D_1 , D_2) again divide vertically. As a result three tiers of four cells each are formed (figs. 21; 22; 23 *e. f.*). The next division of each of these twelve cells is usually periclinal, forming an inner and an outer cell (fig. 24), but occasionally an anticlinal division occurs first, followed by a periclinal one in one or both derivatives, as in the lower quadrant of fig. 25. The outer cell formed by the periclinal division gives rise to the dermatogen in which further divisions are always anticlinal. The inner cell divides by a periclinal division into an outer cell which gives rise to the periblem and an inner cell that forms the plerome (figs. 26, 27).

Returning to the proximal derivative of the supra-median cell (S_1), this frequently divides transversely, but it may divide diagonally (fig. 20) or vertically (figs. 21, 22). In any case it forms a plate of four cells which serves to complete the root tip (figs. 21, 26). Differentiation of the root cap takes place comparatively late, as can be seen in fig. 28, where periclinal divisions of a few dermatogen cells give the only evidence of its formation even in this stage. While the four distal cells of the 8-celled embryo have been undergoing the development just described, the other cells continue to divide in various planes to produce a massive and usually somewhat bent suspensor (fig. 28). A mature embryo, therefore, stands in the following relationship to the cells of the 4-celled stage. The distal cell (D) of the 4-celled embryo gives rise to the cotyledons and the upper part of the hypocotyl; the supra-median cell (S) gives rise to the lower part of the hypocotyl, the root tip, and the upper part of the suspensor; the infra-median and proximal cells (P , I) are concerned only with formation of the massive suspensor.

It has already been mentioned that there are occasional exceptions to the linear condition of the 8-celled stage. One such case is shown in fig. 29, in which the distal cell (D) of the 4-celled stage has divided



FIGS. 28-32.—Fig. 28, longitudinal section of older embryo showing parts derived from each of four distal tiers of 8-celled stage: *Der*, dermatogen; *Per*, periblem; *Ple*, plerome. Fig. 29, longitudinal section of 8-celled embryo derived from 4-celled one in which distal cell divided vertically. Fig. 30, *a, b*, two successive longitudinal sections of embryo derived from one similar to that of fig. 29. Fig. 31, longitudinal section of embryo derived from 8-celled type in which three distal cells have divided transversely. Fig. 32, older stage of embryo derived from one similar to that of fig. 31; $\times 750$.

longitudinally. Although this was the only embryo of this type observed in a great number of 8-celled ones examined, older stages were found which had obviously developed from an 8-celled embryo of similar constitution. For example, in fig. 30, which shows two con-

secutive sections of the same embryo, it is evident that the distal cell

D) of the 4-celled stage first divided longitudinally, and its two daughter cells also divided longitudinally. These four cells then divided transversely to form two layers of four cells each, thus making the end result the same as would have occurred had the distal cell divided transversely in the first place. Evidence for this sequence of divisions is found in the direction of the mitotic figures in one side of the embryo, in the failure of the transverse walls to coincide in the other side, and in the continuity of the longitudinal wall in each section through both distal layers of cells (fig. 30). A study, by means of these criteria, of a great number of 8-celled and more advanced embryos shows that longitudinal division of the distal cell (D) of the 4-celled stage is relatively uncommon. Development subsequent to this stage is the same in this type as in the type described previously.

Although the three distal cells (D_2 , D_1 , S_2) of a linear 8-celled embryo usually divide longitudinally (fig. 15 d), such is not always the case. For example, in fig. 31 there are already 11 cells formed in single file, and two of these are undergoing transverse division, so the embryo will be at least 13 cells long before any longitudinal walls can be formed. This embryo was derived from a linear 8-celled one by transverse division of each of the five cells farthest from the micropyle. The three proximal cells of the eight are still undivided. A somewhat older stage of apparently a similar origin is shown in fig. 32. It seems probable that in this case also, one or more of the three distal cells of the 8-celled embryo underwent transverse division before longitudinal walls were formed. It is to be noted, however, that when longitudinal walls were formed they occurred in the three distal cells only, indicating that the mature embryo will be formed in the usual way from three layers of cells. It seems probable that the three distal cells of fig. 31 will next undergo longitudinal division, and will probably give rise to all of the embryo except the root tip. If this occurs, the embryo will be derived entirely from the distal cell of the 4-celled stage instead of from the distal and supra-median cells, as more frequently happens. Before discussing the significance of embryos of this type it will be well to consider development of the embryo of *Curum* as described by SOUÈGES (17).

In *Carum carvi*, SOUÈGES reports the regular formation of a linear 4-celled embryo similar to that always found in *Daucus*, but finds three different types of 8-celled embryos arising from the 4-celled one. One of these types results from transverse division of each cell of the 4-celled stage, and is similar to the 8-celled embryo usually found in *Daucus*. Another 8-celled type has a longitudinal division of the distal cell and a transverse division of the other three. It is similar, therefore, to the aberrant type of *Daucus* embryo shown in fig. 29. The third type in *Carum* has the distal and supra-median

TABLE I
ORIGIN OF PARTS OF MATURE EMBRYOS OF *CARUM* AND *DAUCUS* FROM
CELLS OF 4-CELLED EMBRYO

CELL OF 4-CELLED EMBRYO	PART OF MATURE EMBRYO DERIVED FROM EACH CELL OF 4-CELLED STAGE		
	Carum	Daucus	
		Usual type	Aberrant type
Proximal	Suspensor	Suspensor	Suspensor
Infra-median	Part of suspensor, root tip	Suspensor	Suspensor
Supra-median	Hypocotyl	Part of suspensor, root tip; lower part of hypocotyl	Suspensor
Distal	Cotyledons	Upper part of hypocotyl; cotyledons	Root tip hypocotyl; cotyledons

cells dividing longitudinally and the other two dividing transversely. No embryo of this type has been found in *Daucus*. SOUÈGES studied the subsequent development of each of these types in *Carum*, and concluded that in all three cases the cotyledons are derived from the distal cell of the 4-celled embryo, the hypocotyl from the supra-median cell, the root cap and part of the suspensor from the infra-median, and the rest of the suspensor from the proximal cell. If this is true, there are some striking differences between *Daucus* and *Carum*. These differences, together with the differences that occur between different individuals of the same species of *Daucus*, are summarized in table I.

It will be recalled that SOUÈGES places much emphasis upon both the arrangement and destiny of the cells in the 4-celled embryo. He states that in *Carum* these embryos are always linear. My observations indicate that the same situation holds in *Daucus*. The studies of HEGELMAIER (8), HAKANSSON (7), and TANFANI (19) show that in a number of other genera of the Umbelliferae linear 4-celled embryos are regularly produced. The other conclusion of SOUÈGES, that within the family the destinies of the cells of the 4-celled stage are usually the same, is not supported by this study of *Daucus*, as shown in table I. This table shows that the parts of the mature embryo of *Carum* do not originate from the same cells of the 4-celled embryo as do those of *Daucus*. SOUÈGES' figures show that there is a possibility that he has misinterpreted the method of origin of certain of the advanced stages. If this proves to be the case, the development of *Carum* will be found to be the same as that found most frequently in *Daucus*. The table also shows, however, that each cell of the 4-celled *Daucus* embryo does not always give rise to the same part of the mature embryo. It is of interest to note that these aberrant types are of such a nature that they increase rather than decrease the differences between *Daucus* and *Carum*. The conclusion of SOUÈGES, that the destiny of the cells of the 4-celled embryo is rather constant throughout a family, obviously does not hold in the Umbelliferae, because in this family we find differences not only between different genera but between individuals of the same species. Until more genera have been investigated it will be unsafe to attempt any generalization as to the manner in which the cells of the 4-celled stage contribute to the mature embryo in the Umbelliferae.

Summary

1. This paper is concerned with the development of the female gametophyte, the pollen tubes, and the embryo of *Daucus carota*.
2. A single archesporial cell functions directly as a macrospore mother cell which produces a linear tetrad of macrospores.
3. The megagametophyte, which arises from the chalazal macrospore, is of the 7-celled type characteristic of most angiosperms.
4. The presence of a cellulose filiform apparatus was demonstrated by microchemical tests.

5. The structure and path of the pollen tubes were investigated. Tubes were found to grow intercellularly down through the conducting tissue of the style to its base, and then superficially along a groove leading to a canal communicating with each locule. Tubes growing down one style may enter the locule immediately below or grow through the transverse canal and into the other locule.

6. A filamentous 8-celled embryo is usually formed before longitudinal divisions occur. The three cells farthest from the micropyle give rise to all of the embryo except the root tip. The other five cells give rise to the root tip and the suspensor.

7. Development of the embryo was considered in the light of SOUÈGES' theory that the 4-celled embryo is the critical stage in development, and that for any given species the four cells always give rise to the same parts of the embryo. In *Daucus* the mature embryo may arise entirely from the distal cell of the 4-celled embryo, or from the distal cell and derivatives of the cell next to it. SOUÈGES' further generalization that embryonal development is usually characteristic throughout a family does not appear to hold in the Umbelliferae, as is shown by a comparison of *Daucus* with his account of the embryogeny of *Carum*.

The author takes this opportunity to thank Dr. GILBERT M. SMITH for his assistance during the preparation of this paper.

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[Accepted for publication August 13, 1930]

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UNUSUAL ASPECTS OF MEIOTIC AND POSTMEIOTIC CHROMOSOMES OF GASTERIA¹

HSE-CHUAN T'UAN²

(WITH PLATES I, II AND FOURTEEN FIGURES)

Introduction

The investigation here described was initiated at the University of Pennsylvania in 1929. The preliminary work consisted of a study of the comparative action of different fixing reagents and of the methods of applying different nuclear stains. The investigation covered a wide range of plant material.

The technique of making cytological smears (20) rendered the question of fixation comparatively simple. Emphasis was therefore placed upon the acquiring of a satisfactory staining technique. It was found that picric acid destains material which has previously been treated with haematoxylin and safranin, and by its use the writer was able to get consistently satisfactory results. *Gasteria* was first investigated, and subsequently smears of *Clarkia*, *Solanum*, *Pinus*, and many others were studied experimentally. In the course of this study, a group of *Gasteria* smears, showing microsporogenesis, yielded an unusual phase of diakinesis chromosomes. This discovery led to the present report. In diakinesis, some cells showed a sudden change in their capacity for absorbing the stains, and in addition they were found to go through a regressive process to a false resting stage. Often the pollen mother cells of this particular plant divide three successive times, forming eight pollen grains.

TAYLOR (21, 23, 26) had previously made a thorough study of *Gasteria* chromosomes, and contributed much to our knowledge of their shape and structure. A comparable investigation of this series of abnormal chromosomes in the same genus might therefore be expected to reveal other interesting facts.

¹ Contribution from the botanical laboratory of the University of Pennsylvania.

² Fellow of the Chinese Educational Mission at Washington, D.C., of Tsing Hua University, Peiping, China.

A part of this work was done at the Marine Biological Laboratory, Woods Hole, in the summer of 1930, after the material had been collected and partially studied at the University of Pennsylvania. The research was then completed at the botanical laboratory of the University of Michigan. To these institutions the writer is much indebted for laboratory space and the necessary equipment.

Material and method

Pollen mother cells of *Gasteria* were collected in a greenhouse at the University of Pennsylvania in the spring of 1930. Young buds were teased out with a needle and the anthers transferred immediately to a thoroughly cleansed slide. With a stroke of a scalpel, the pollen mother cells were smeared evenly into a thin film on the slide, which was then submerged in the fixing fluid for 20 minutes. Many fixing fluids were tried, and although the results varied much in detail, the fixation of smears was generally better than preparations sectioned by the paraffin method. The following account is based upon the result obtained by the use of a special chromic-acetic-osmic fixative developed by TAYLOR (McCLUNG 11) for *Gasteria* pollen mother cells. No paraffin preparations were used, since the smears give the most natural image of the cell, both in regard to its general contour and to the details of its chromosomes. Shrinkage is almost eliminated in most of the cells. The staining technique described by TUAN (27, 28) was followed, with some slight modification to suit each individual case. When haematoxylin was used, the picric acid differentiation was occasionally abbreviated by the slides being placed in a 0.5 per cent iron alum solution for 2 minutes before they were transferred to the picric acid for further differentiation. This saves the long schedule of differentiation required when picric acid alone is used. For *Gasteria*, however, the writer still prefers the long schedule of picric acid, but in the case of *Tradescantia*, prepared for purposes of comparison, a previous treatment in a 0.5 per cent iron alum solution cut down the time from 3 hours to 30 minutes. When stained with safranin, the slide was brought into a mixture of two parts of clove oil and one part of 95 per cent alcohol, and there the differentiation was further adjusted. Deeply stained or over stained slides can be saved by this process.¹ One example

¹ This alcohol-clove oil differentiation can also be applied to crystal violet smears.

from each of the methods illustrates their general application to the *Gasteria* study:

SLIDE PK 8, SAFRANIN	MINUTES
1. Stained in Grubler's safranin O	5
2. Picric acid-alcohols	5
3. Ammonia-95% alcohol	o 5
4. Pure 95% alcohol	o 5
5. 95% alcohol-clove oil mixture	5-10
SLIDE KP 9, HAEMATOXYLIN ¹	
1. 2% iron alum	20
2. $\frac{1}{2}$ % haematoxylin	5
3. Differentiated in picric acid	160
SLIDE KP 6, HAEMATOXYLIN	
1. 2% iron alum	20
2. $\frac{1}{2}$ % haematoxylin	5
3. $\frac{1}{2}$ % iron alum	2
4. Differentiated in picric acid	30

Investigation

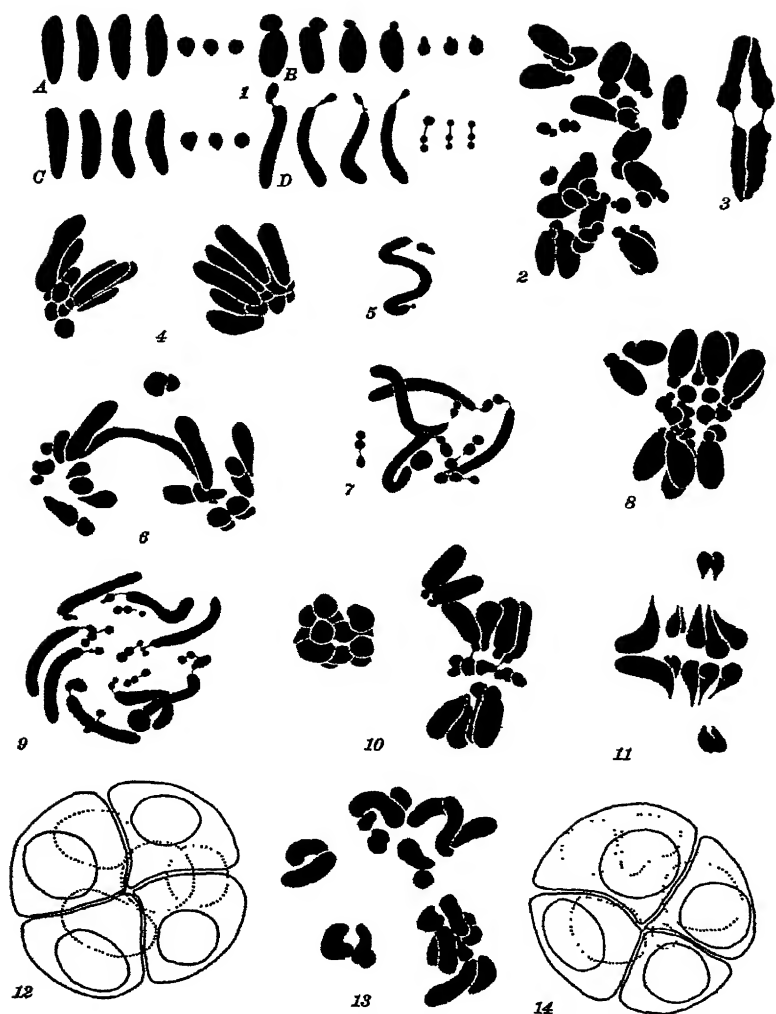
In the normal behavior of *Gasteria* chromosomes, according to TAYLOR (21, 23, 26), the first suggestion of prophase activity in meiosis is marked by an aggregation of chromatic granules, forming more or less spiral chromonemata. When the spirals straighten out from a tangled condition to form the extended chromatic threads, synapsis of homologous chromosome pairs occurs simultaneously at different places throughout the nucleus. The longitudinal split of the paired chromosomes, which form the tetrad, is accomplished before the diakinesis stage. Three pairs of pyriform chromosomes and four pairs of larger chromosomes are present on the metaphase plate. The tetrad condition of each pair is revealed distinctly. A coiled spiral of from seven to nine turns is present in the longer chromosomes, while in the small ones the number of coils is correspondingly reduced. These coils apparently are a result of the contraction of the more or less straight chromonemata of an earlier stage. In the anaphase, the two chromatids of each dyad may separate and the coils within them become more distinct. When telophase is reached, the chromosomal sheath is no longer stainable but

¹ When haematoxylin is used, each stage is followed by a prolonged washing in tap water.

is still present. Eventually the chromosomes become disorganized in the interphase. When they reappear for the second division, their shape and internal structure acquire interest from a new angle. At the proximal end of each chromosome a mild constriction is invariably present; perhaps in the three short ones a secondary constriction occurs along the shaft. In size, the homeotypic chromosomes are more slender and longer than the heterotypic ones. The internal structure of each of the homeotypic chromosomes resembles that of the somatic chromosomes, that is, each chromosome contains two chromonemata derived from a split of the coils. A quartet is formed as a result of two cell divisions. Each of the resulting cells divides again after the formation of a thick wall, typical of pollen grains.

There are several striking differences between the normal behavior of *Gasteria* chromosomes, as just described, and the peculiar mode of division to be recorded in the next few paragraphs.

The first sign of abnormality is a dispersal of the diakinesis chromosomes to form a false resting stage. More complicated abnormalities occur after the first metaphase. The nuclear membrane fails to form at telophase. Two sets of second metaphase chromosomes persist through the omission of the interphase and second prophase. The chromosomes thus produced assume two different aspects. The type (fig. 30) with distinct spiral structure is identical with that which appeared in the first metaphase; the other type (figs. 2, 8, 10, 18) is erratic and cannot be connected with any particular division of the chromosomes. So far as their outer form is concerned, they are intermediate between the homeotypic and heterotypic chromosomes, as they have extraordinary terminal heads and short, heavy bodies. Abnormal chromosomes of another type (fig. 9) are produced by some cells in which a nuclear membrane is formed after the telophase. The probable fate of these last was not determined, owing to a lack of sufficient material of this type, but the history of the chromosomes with spiral structure is traced to the formation of third division chromosomes within the nucleus of quartet cells (fig. 71). No regular division of the quartet was observed, but furrows of various depths occur in these cells, and have been noted in many preparations. This suggests that the third division is carried through by a simple process of furrowing. On the



FIGS. 1-14.—Fig. 1, selected chromosome sets showing four types of chromosomes: *A*, first metaphase chromosomes; *B*, second metaphase chromosomes with extraordinary heads; *C*, second metaphase chromosomes with shape and appearance of first metaphase chromosomes retained; *D*, set of constricted chromosomes. Fig. 2, second metaphase figure showing giant headed chromosomes scattered irregularly in cytoplasm; 28 elements counted in all, but only 26 shown. Fig. 3, pair of anaphase chromosomes showing bulging matrix. Fig. 4, early telophase showing partial rotation of daughter groups. Fig. 5, one of constricted chromosomes from binucleate cell showing satellite at lower end. Fig. 6, mid-anaphase figure showing partial rotation of daughter groups and non-separation of single chromatid. Fig. 7, third division chromosomes with all types of constrictions represented. Fig. 8, group of second metaphase chromosomes showing giant headed chromosomes and extruded dyad. Fig. 9, constricted chromosomes from binucleate cell. Fig. 10, two sets of giant headed chromosomes showing extruded dyad at periphery. Fig. 11, extrusion of small chromosome at equatorial plate stage. Fig. 12, 8-celled polyspore. Fig. 13, second metaphase figure showing extrusion of dyad from each of two sets of chromosomes. Fig. 14, 9-celled polyspore.

other hand, these chromosomes, like some of the diakinesis elements, may disintegrate and produce false resting nuclei.

REGRESSION OF CHROMOSOMES FROM DIAKINESIS

The clearest evidence of tetrad chromosomes was observed during the diakinesis stage (fig. 16). From the general appearance of these threads, it seemed that the cell was perfectly normal, and had behaved as described by TAYLOR. The only peculiarity up to the first metaphase was a suggestion of disintegration of the thickened diakinetid chromosomes, which were recognized in a few cells by the fact that they stained faintly (fig. 17). When these chromosomes are traced through several stages, it is found that they collapse completely. The tetrad is gradually brought clearly into view with the homologous chromatids connected by numerous achromatic bridges (fig. 32). The chromosomes may be compared to a rope ladder. In a later stage the achromatic bridges are replaced by a clear space, so that parallel threads of an early prophase nature are formed (fig. 29). Further disintegration of the threads brings about a false resting stage, which might be mistaken for the earliest evidence of the first prophase, except that the nucleolus in this case is absent. Probably cells of this type will not divide again, as there was no evidence to show a reorganization for further division.

Those cells which show the same kind of disintegration but at a later stage will not be described in detail. A brief account of their development, however, may bring out some points for discussion. (1) Empty cells are frequently observed among all stages of meiotic figures. (2) Cells at the binucleate stage or quartet stage which show only a large but faintly stained nucleus are also frequent. (3) Nuclei of a quartet may show different stages of activity (fig. 40), sometimes from one to three nuclei of a quartet being missing. Whether these cells represent a complete breakdown of the chromatin material or not could not be determined, but from this example of the diakinetid chromosomes it seems likely. As to the cause of these abnormalities, it is rather rash to connect them with any one agent, such as pathological influence or genetical unbalance. It is most probable that there were other forces which, in addition to these, contributed much to the peculiar behavior of these cells.

FIRST METAPHASE.—The lapse of time from diakinesis to metaphase is exceedingly short. This observation is confirmed by a general lack of intermediate stages in many preparations, although in a few cases figures of such were not hard to identify. When diakinesis ends, the chromosomes shorten and thicken with surprising rapidity. The scattered chromosomes are suddenly gathered up in the central portion of the cell, while the nuclear membrane disappears entirely and the fiber attachments are formed on each of the chromosomes. It seems that the chromosomes which are now not in contact with the cytoplasm are able to take up the stains more readily than at any other stage; therefore the metaphase chromosomes arranged on the equator have the appearance of solid rods, and their internal structure is detected only in well differentiated preparations. On the metaphase plate, seven pairs of chromosomes, split into tetrads (fig. 27), can be observed so far as the general contour of the cell and the chromosomes are concerned. There are, however, several striking cases of abnormal distribution. In a few cells one of the small chromosomes is found at the polar region when the rest of the set is still on the equator (fig. 11). Also, one of the small chromosomes is often extruded entirely from the spindle region (fig. 6), so that when migration is completed, it is left at the peripheral region of the equator where it rounds up quickly to form a small nucleus.

FIRST ANAPHASE.—The anaphase chromosomes can clearly be counted with seven dyad chromosomes in each daughter set. At the beginning of migration, the chromatids are evidently subjected to a strong pull between the spindle fibers and the surfaces of contact with their corresponding homologues. When they are midway between the pole and the equator, they are stretched to a certain extent, the turns of the spiral bulging out at places and suggesting the presence of an elastic matrix (fig. 3). Slender threads may be seen between them as the daughter chromatids are drawn farther and farther apart. As a rule, the partners of each dyad are separated before they reach the pole (fig. 31). In one extreme case (fig. 6) one of the chromatids was pulled across the equator and failed to separate. The telophase (fig. 4) follows, with a general lack of the compactness of the chromosome such as is noticed in the normal condition. The small chromosomes reach the pole ahead of the large

ones, although migration started simultaneously in all. Many cells now pass quickly to a second division without any of the phenomena associated with the heterotypic telophase. Only the least abnormal types, those which retain some evidence of telophase dispersal, will next be considered. However, a brief partial telophase may result in the formation of chromosomes as seen in figs. 2, 8, 10, and this will be described in connection with the second metaphase chromosomes.

RESIDUAL FIRST TELOPHASE AND DERIVED CHROMOSOMES

The ends of the chromosomes toward the polar region approach each other, but the distal ends, extending toward the equator, alter their position very little. This is soon followed by a gradual rotation of the two groups of chromosomes in opposite directions (figs. 4, 6). After a complete turn of 90° they lose their staining ability. There are a few examples of nuclear membrane formation, but the majority of the cells do not show such a tendency. The former type of nuclei lead to another abnormal division, while the latter (fig. 24) soon recover their staining properties, so that they are seen as full sized chromosomes on the second metaphase plate (fig. 30). The interphase and second prophase of the latter type of nuclear division are entirely skipped, and consequently the appearance and structure of the chromosomes are unchanged from the first division, a condition similar to that of some animals in which the interphase and prophase are skipped during maturation divisions of the egg nucleus after entrance of the sperm.

In those cells in which the nuclear membrane plays a part, the chromosomes show a progressive loss of staining ability. The membrane appears first at one side, usually the side near the polar region (fig. 21), whence it grows about the mass to complete its formation (fig. 19). At this stage, as the nucleus increases in size, the chromatids are so lengthened that a continuous but loosely coiled spiral is seen in each (figs. 19, 21). Further stretching of the spirals (fig. 20 *a, b, c*) gives rise to an isolation of a single loop at the proximal end of each chromosome. Later the nuclear membrane, perhaps as a result of the excess lengthening of the chromosomes, is ill-defined and suggests that it is in the process of disintegration. Further evidence derived from the formation of heavily constricted

chromosomes (fig. 9) would seem to indicate that the nuclear membrane is finally and entirely disorganized. The cause of such a phenomenon was not determined, but it is interesting to note that immediately after disappearance of the membrane, two sets of constricted chromosomes are found in the cell. The origin of this type of chromosomes is probably that as soon as isolation of the tips of the spirals is accomplished (figs. 20, 23), anastomoses appear (figs. 37, 38). At first they are difficult to recognize, but later they assume a distinct threadlike form (fig. 9). This process is accompanied by the vacuolation characteristic of telophase chromosomes. The nuclear membrane is indefinite, and its complete collapse initiates reorganization of the chromosomes. A count of the elements in each of the cells reveals fourteen as the number (fig. 23). Two of them bear extremely big heads, which are further divided by another contraction (fig. 9). Six of them show comparatively small heads, while each of the six small chromosomes is terminated by a round head and has also a median constriction. All of these heads can be mistaken for satellites, as they are connected to the shafts by very slender threads. In one case, one of the large chromosomes showed a small satellite at the distal end (fig. 5). Presence of true satellites in meiotic chromosomes has not yet been reported, and their purely vegetative nature was emphasized by TAYLOR (23). No inference can be drawn from this sole exception.

SECOND METAPHASE.—It has already been mentioned that the interphase and prophase are skipped in many of the cells, owing to failure of the nuclear membrane to appear. This account of the behavior of these cells will therefore begin with the metaphase of the second division.

The chromosomes which retain the form they had in first division telophase have now recovered their staining ability in a surprising manner (fig. 24), each of the two sets of metaphase chromosomes being arranged on a second equator for the following division (fig. 30). The plane of this division is perpendicular to that of the first, as is shown by the rotation of the daughter groups in the earlier stages. From these they can clearly be seen as two sets, not arranged in a definite pattern. No case could be found in which the chromosomes were not widely separated from one another; this is

perhaps one of the reasons that a lagging and an extrusion of chromosomes will later occur. Rejection of a dyad chromosome was frequent, while the elimination of a dyad or two (fig. 13) from each daughter set of chromosomes was common. Those eliminated may give rise to some supernumerary spores.

Another type of abnormality is found at the second metaphase plate. Although many chromosomes show an internal spiral structure, there are some cells whose chromosomes show a striking difference (figs. 2, 8, 10, 18). These latter are constricted, with extraordinary heads at the tip, while the body is of a peculiar type, short and heavy. By an analysis of all the figures available, the origin of these chromosomes was traced back to the first telophase, when the nuclear membrane was just about to form. Consequently it is most probable that at telophase the nuclear membrane of these cells has a very brief existence, so that the chromosomes are only partially disorganized for the homeotypic division. Further collapse of the spindle would undoubtedly give rise to these scattered chromosomes (fig. 2). In fig. 2 the total count of the chromosomes is 28, but only 26 can be shown in the drawing. The count of 28 is exactly four times the haploid number. From the size of the heads, it is further determined that the total complex represents four sets of chromosomes, and each set (fig. 1 *b*) has the haploid number of seven. For these chromosomes at least, it shows that the chromatids of each tetrad are completely separated at late anaphase. Identified by the heads, these chromosomes are traced to the resting quartet stage. Late anaphase cells are frequently seen with four sets of chromosomes spread widely in the cytoplasm (fig. 26). Disintegration of the chromosomes, typical of telophase activity, occurs. Formation of a nuclear membrane is postponed until disintegration of the chromosomes is almost completed (fig. 33 *a-c*).

SECOND ANAPHASE.—The second early anaphase is brief. Cells are often observed in late anaphase (fig. 25) and telophase. It has been stated that the metaphase chromosomes are loosely scattered along the equatorial region, therefore it is to be expected that various abnormalities of the chromosomes would appear in the second anaphase. Several cases of chromosome extrusion from the spindle regions have been mentioned in connection with the metaphase chro-

mosomes, but as the appearance and history are similar to what has been described, a repetition will not be attempted.

SECOND TELOPHASE AND THIRD DIVISION CHROMOSOMES

In many cells, when the chromosomes have reached their respective poles, they round up to form the telophase. Ultimately a regular resting quartet is formed, all the chromosomes having lost their individuality within the nuclear membrane. Probably the meiotic activity of the cells developing in this manner is thereby concluded. There are a number of quartets, however, which retain the spirals throughout the telophase in perfect shape until a new set of active chromosomes is formed (fig. 7). Although these chromosomes were not observed to go through an elaborate division, their formation in the quartet nucleus is sufficient evidence for such a possibility. The history of the spirals here seems to be of the same nature as that shown in the first telophase, although the number in this case is reduced one-half. Furthermore, the constricted chromosomes formed after second telophase (fig. 9) are identical with those found after the first telophase. The development of these chromosomes could be only partially traced. It may be said, however, that at the end of the second telophase, the chromosomes gradually lost their chromaticity as the coiled spiral reappeared (figs. 28, 39). Each of the spirals then elongates at the expense of its width, so that the coils become very narrow compared with those first involved in this process. A loop or two at the tip of each spiral is then gradually dragged apart from the main body as the chromosomes reach their maximum length. The chromosome number is seven: one bears a big head (fig. 1 *d*), three are terminated by smaller heads, while at the tip of the small chromosomes a round head is shown with a secondary constriction along the shaft. This combination is exactly like that found after the first telophase, except that each type is represented by a single element. There is not much detail to be seen in these chromosomes. Their structure was discerned, however, in other cells in which the chromosomes were undergoing a process of regression. They had a single spiral. These chromosomes resemble the homeotypic ones in their constrictions, but they are different in structure, as the homeotypic chromosomes under normal condi-

tions have two spirals in each. The question arises as to whether or not this type of abnormality should be connected with the normal homeotypic chromosomes. The writer will develop this point later.

Several interesting figures may be observed in the regression quartets. The quartet nuclei in many cases show different stages of activity in the same group (fig. 40 *a-c*). A typical resting nucleus is shown in *b*; *c* is shown with partially disintegrated chromosomes; while *a* is intermediate. From the equal sized and equally stained nucleoli in these cells, it is probable that these stages are passed through in a short period, and the probable disturbance occurs immediately after formation of the third division chromosomes. Reformation of the nuclear membrane later will mark the beginning of the regression process. The most probable steps of chromosome behavior in these cells are as shown in fig. 22 *d, c, b, a*, and figs. 36, 40. The earliest stage of disintegration is marked by a loss of chromaticity of the chromosome matrix, so that the comparatively heavily stained spiral within the chromosomes is brought out in better contrast (fig. 22 *c*). The chromosome at the lower right hand corner of fig. 36, and two other segments in the same cell, show the spiral clearly, while the farther ends of these chromosomes are in a more advanced stage of disintegration. Increasing number of anastomoses and central vacuolation (fig. 22 *a, b*) bring the chromosomes close to a resting stage (fig. 40 *b*). It seems that the chromaticity of each spiral varies at regular intervals, and usually certain segments are still heavily stainable while others register little affinity for the stain (fig. 40 *a*), so nodules are distinctly shown at places throughout the nucleus. When vacuolization reaches a climax, the chromosomes appear double, with faintly stained peripheral regions and clear central cylinder (fig. 40 *b*). Besides these regression figures, there are two selected from a number of quartet cells worth mentioning. The one (fig. 34) with short spirals is rather difficult to assign to a particular developmental position. It is probably a second telophase figure, however, with the spirals segmented into short pieces. The other (fig. 35), with slender but continuous spirals, has been given no definite position either. There are four longer spirals and three shorter ones. If it is not one of the second telophase figures, it must be classified with the regression chromosomes, and should be followed closely by the spirals shown in fig. 36.

POLYSPORY

In several preparations, 8-celled spore groups are found among other abnormal groups containing from five to many cells in place of the normal quartet. It has been suggested that the lagging of chromosomes at first metaphase and at many other stages may produce extra nuclei, therefore all the supernumerary cells in any of these groups may be considered to be the natural consequence of such abnormalities. A detailed consideration of the 8-celled groups strengthens the assumption that third division does occur in some of the cells.

Eight nuclei of equal size frequently occur in 8-celled groups (fig. 12), while one or two small extra nuclei (fig. 14) are also frequently present. It is easy to account for the small nuclei as the result of misplaced chromosomes, but as to how the eight equal sized nuclei developed, there is only one reasonable explanation; that is, that they are the product of a third division. The evidence in support of the presence of a simple third division may be summed up in a few words. As soon as the chromosomes are formed in each of the four nuclei of a quartet, the nuclear membrane disappears, although the nucleolus, formed at the end of the telophase, is still obvious (fig. 7). The chromosomes, usually seven in number, spread apart in the cell without sign of spindle formation. The next stage, thus far observed, involves cleavage of various depths along the cell wall and cytoplasm. Whether this is the mode of the third division or not is without further proof, and the method by which these chromosomes are distributed to the daughter nuclei is another unanswered question. Additional work on the pollen mother cells of this plant may bring to light more evidence.

RÔLE OF NUCLEOLUS

From the resting cells up to the diakinesis stage, the nucleolus remains in the nucleus with little change. Its diameter is estimated as $2.5-3.5\mu$ (figs. 15, 16). It disappears almost simultaneously with the nuclear membrane as the chromosomes are arranged for the first division metaphase. It is observed again as a faintly stained body at the end of the first telophase, with a much reduced diameter ($1-1.5\mu$). It increases in size within the nucleus containing the first type of constricted chromosomes. At this stage it measures

about $2\ \mu$ in diameter (fig. 9). With the other type of division, where the interphase and prophase are omitted, the nucleolus does not appear until the second telophase is far under way. It shows the same diameter as before; that is, it first appears as a body about $1\ \mu$ across, and then increases in bulk when the third division chromosomes are formed (fig. 7). Its definite function in this process cannot be accounted for; however, the interpretation that the nucleolus has some connection with the chromosomal appendages is eliminated. In this case it occurs simultaneously with the fully developed chromosomes, therefore it is not converted into any of these appendages.

CHROMOSOME STRUCTURE

Since the structure of chromosomes has already been discussed, only a few outstanding points will be added here. On the metaphase plate the chromosomes are heavily stained. From haematoxylin preparations, the spiral structure is not very distinct, but the number of coils was easily ascertained by the nodule-like structures which bulged out at places along the elastic matrix. It is estimated that in the longer chromosomes the spiral has from seven to nine turns, while in the small ones there are only two or three present. In many metaphase cells indication of a longitudinal split was not observed. The single nature is retained throughout until the formation of chromosomes with satellite-like appendages. As previously mentioned, this type of chromosome, in its outward appearance, resembles the normal homeotypic chromosome, but from its internal structure the resemblance is entirely lacking. The cause for such difference is probably due to the fact that the prophase, in which the split is supposed to take place, was skipped, and therefore the split was perforce omitted. The significance of this process is not understood, but it confirms the interpretation of TAYLOR (26) that the double spiral in the homeotypic chromosomes is the consequence of a longitudinal split completed at the second prophase. The internal structure of those chromosomes with giant heads is rather obscure. In many well differentiated cells, it is seen as a comparatively lighter central core with a densely stained peripheral region (fig. 18), containing well spaced nodules in two rows, a structure which makes the chromosomes appear double. When the chro-

mosomes come to the second anaphase (fig. 26) the same kind of apparent doubleness is maintained, in addition to the vacuoles and anastomoses.

Discussion and historical review

Several diverse problems are involved in the abnormal behavior of *Gasteria* chromosomes: (1) the occurrence of three successive divisions in microsporogenesis; (2) the appearance of the heterotypic type of chromosomes in the second division; (3) the exaggerated constrictions of the chromosomes; (4) the production of polypores after the close of meiotic activity; (5) the regression of chromosomes from different stages of chromosomal development to a false resting stage; and (6) the structure of chromosomes.

Literature recording three or more divisions at the time of meiosis is scattered. Both in spore formation in Ascomycetes and oogenesis in Fucaceae, as well as in embryo sacs, it is well known that mitoses may occur after meiosis before the full number of cells is completed. The occurrence of the division at very different periods in the morphological life cycle suggests that the divisions resulting in polypory are not unforeshadowed and altogether new in higher plants. Recently Stow (18), by applying a higher temperature to growing bulbs of *Hyacinthus*, obtained multinucleate giant pollen grains. He found that cells dividing three times in succession produce structures resembling embryo sacs, while those dividing four or five times give the same kind of irregularity but of a more complicated form. He concluded that the male potency in these cells is reduced by the physical means employed, and thus the female tendency is given a temporary dominance. Although this conclusion is hardly applicable to this case of *Gasteria*, where signs of embryo sac formation are entirely lacking, it suggests the possibility that material for this study was injured by some similar agents in the greenhouse before fixation.

As to the appearance of a similar type of chromosomes in the first and second division during meiosis, no corresponding case can be found among plants. Among certain forms of animals such a condition is of common occurrence, therefore it is rather hasty to establish any co-relation here.

Constrictions and satellites have been reported frequently as a

permanent morphological character for individual chromosomes. TAYLOR (21, 22, 23, 24, 25) identified certain chromosomes in many forms by the constrictions and satellites. SVESCHNIKOVA (19) classified the genus upon the size of chromosome heads and number of satellites seen in many species of *Vicia*. SAKAMURA (15) and many others, by means of the use of chloral hydrate and by following the constrictions, have settled the disputed counts in several plants and corrected the old conception of chromosomal tetrads and transverse divisions. However, the origin of such morphological characters is only partially discussed in these articles. NAWASCHIN (12) probably contributed the first interpretation that nucleolar material is responsible for the satellites. TAYLOR (22) and DARLINGTON (3) claimed that the satellite is the natural result of an extreme type of subterminal constriction. In the present case, it is seen that the satellite-like appendages are the direct production of isolated loops of chromatic spirals. Their coexistence with a nucleolus in many of the cells makes it obvious that the nucleolar material of *Gasteria* plays no direct part in the formation of such appendages.

There is an enormous amount of publication on polyploidy. Almost without exception each article deals with hybrids or polyploid species. Taking the rose family for example, ERLANSON (4), in a study of American wild roses, found that polyploidy is a common phenomenon among spontaneous hybrids or descendants of hybrids; SHOEMAKER (17) arrived at the same conclusion with species of cultivated apples; LONGLEY (9, 10) discovered the stability of diploid species in *Crataegus* and *Rubus*, while "The polyploid species are characterized by striking irregularities in chromosome distribution and irregular pollen-formation, leading frequently to polycary and polyploidy." Investigations on *Potentilla* (14) and many related genera indicated the same results. In *Gasteria*, TAYLOR (21) has indicated the hybrid origin of much of the material in cultivation, and questioned the authenticity of specific names as commonly used. There remains little doubt that the species studied is by no means pure, and perhaps some of the abnormalities can be explained on account of genetic weaknesses.

In connection with the regression of chromosomes, experiments

by CHAMBERS (2) are especially interesting. He demonstrated by microdissection that the reversion of jelly-like chromosomes to a more or less homogeneous sol could be brought about by mechanical injury. With fixed material, degeneration of quartet nuclei is described in several cases. In *Melilotus alba*, ROGERS (13) and CASTETTER (1) reported disintegration of three nuclei in quartets and the formation of giant pollen grains by the fourth nucleus. In *Carex* the same condition of disintegration of certain quartet nuclei and giant pollen formation is given by JUEL (5). From these observations it may be inferred that disintegration of nuclei is a normal phenomenon in certain species, and in some others it may be the result of mechanical injury.

KAUFMANN (6, 7), KUWADA (8), SHARP (16), and TAYLOR (26) have already offered lengthy reviews on the structure of large chromosomes. The discovery of spiral chromonemata and interpretation controverting the older investigators have also been recorded many times. It now seems that the structure of the larger chromosomes is understood to a degree. What remains to be investigated is the structure of smaller chromosomes. With the aid of new and more accurate technique, it is certain that many of the observations of larger chromosomes will be linked together into a coherent interpretation when the smaller chromosomes begin to attract the attention of the more skilled workers.

So far as behavior of the chromosomes is concerned, the peculiar cases of *Gasteria* recorded here are exceedingly interesting as well as perplexing. In their natural environment, such unusual behavior is perhaps being reported for the first time. However, judging from the abnormalities induced by chloral hydrate (15), excessive heat (18), and mechanical injuries (2), it is possible that some physiological factors are involved. On the other hand, it is probable that the material used is from a certain hybrid species of *Gasteria*, as shown by the polyspory and lagging of chromosomes at different stages of chromosomal development. It would be interesting from both physiological and cytological points of view, therefore, to conduct some parallel experiments with well controlled factors, whereupon it would not be difficult to learn some of the causes of the disturbance.

Summary

1. Behavior of normal reduction chromosomes of *Gasteria* as described by TAYLOR is cited and summarized.

2. Three types of abnormal meiotic and postmeiotic chromosomes of the same plant are described. One type, represented by closely coiled second metaphase chromosomes, is directly derived from the heterotypic chromosomes by an omission of the first interphase and second prophase. The other type, represented by giant headed chromosomes, is derived from the first metaphase chromosomes through a partial telophasic disorganization. The third type, represented by heavily constricted chromosomes in binucleated cells, is derived from the first metaphase chromosomes through a short lived interphase and breakdown of the nuclear membrane. The constrictions are much exaggerated and resemble the satellites in the somatic chromosomes.

3. The spiral which is involved in the structure of these types is single through the omission of the first interphase and second prophase.

4. Formation of third division chromosomes and 8-celled groups is described. This condition is thus far unique in higher plants.

5. Abnormal diakinetid chromosomes and chromosomes at other stages are able to assume a false resting stage after a brief process of disintegration of the chromatic material.

6. A nucleolus was seen in nuclei with fully developed chromosomes; it was not converted into any of the chromosomal appendages.

It is a pleasure to acknowledge the guidance of Professor WM. RANDOLPH TAYLOR, under whose supervision this study was conducted. The writer also wishes to express his hearty thanks to Professor CONWAY ZIRKLE and Dr. JOHN M. FOGG Jr. for their criticisms and suggestions regarding the manuscript.

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[Accepted for publication December 15, 1930]

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EXPLANATION OF PLATES I, II

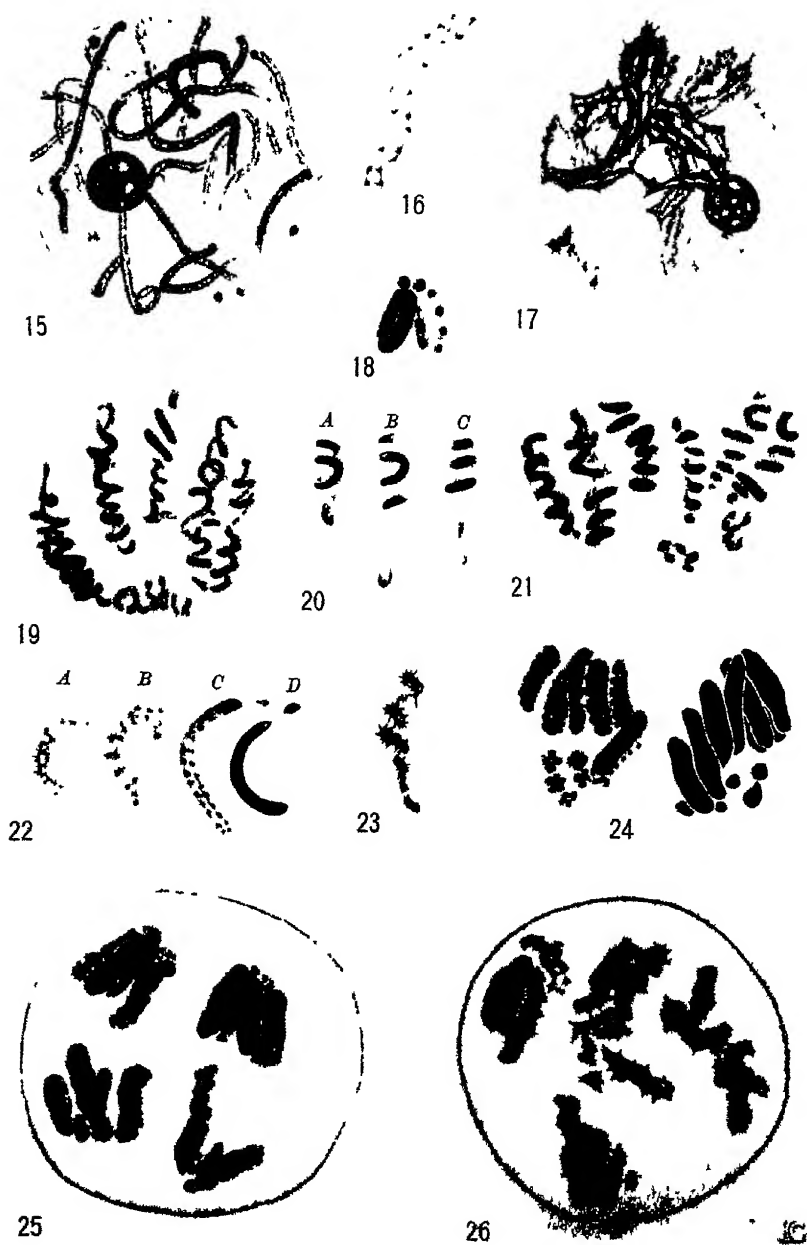
All drawings were made with the aid of Zeiss 1.5 mm. apochromatic objective and $\times 20$ compensation ocular, giving an approximate magnification of 4500.

PLATE I

- FIG. 15.—Open spireme nucleus.
FIG. 16.—Nucleus at diakinesis stage.
FIG. 17.—Segment of diakinetid chromosomes showing first sign of regression.
FIG. 18.—Giant headed chromosomes showing structural detail.
FIG. 19.—Mid-telophase chromosomes showing loosely connected spirals.
FIG. 20.—First telophase chromosomes (*a*, *b*, *c*) showing origin of constrictions.
FIG. 21.—Early telophase nucleus showing loosening of spirals and formation of nuclear membrane at side near polar region.
FIG. 22.—Third division chromosomes (*a*, *b*, *c*, *d*) showing process of regression.
FIG. 23.—Late telophase spiral showing anastomoses and vacuolation.
FIG. 24.—Two sets of spiral chromosomes just before second metaphase.
FIG. 25.—Second anaphase of spiral chromosomes.
FIG. 26.—Second anaphase of giant headed chromosomes.

PLATE II

- FIG. 27.—First metaphase chromosomes at equatorial plate stage.
FIG. 28.—Spirals in quartet nucleus, seven can be counted.



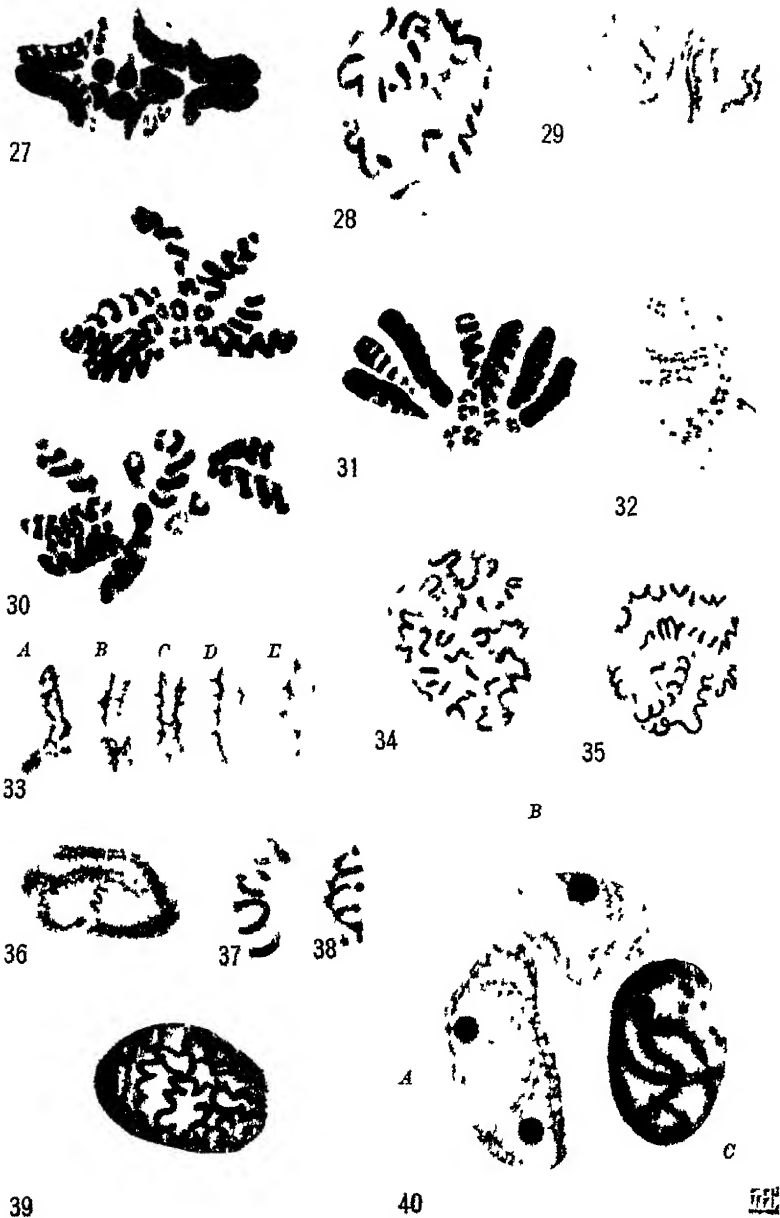


FIG. 29.—Chromatic thread from regression diakinetid chromosome.

FIG. 30.—Two sets of second metaphase dyads derived directly from first metaphase tetrads showing spiral structure in each of chromatids.

FIG. 31.—Late anaphase daughter group showing separation of dyad partners.

FIG. 32.—Tetrad threads from regression diakinetid chromosomes.

FIG. 33.—Selected second telophase giant headed chromosomes (*a, b, c, d, e*) showing disorganization of chromatin material; head still recognizable in *a*.

FIG. 34.—Spirals from quartet nucleus.

FIG. 35.—Seven continuous spirals from another quartet nucleus.

FIG. 36.—Quartet nucleus showing regression of third division chromosomes.

FIG. 37.—First telophase spiral showing anastomoses.

FIG. 38.—Same as fig. 37.

FIG. 39.—Quartet nucleus at early telophase, seven spirals can be counted.

FIG. 40.—Same as fig. 36. *b* shown at resting stage.

DISCHARGE OF SPERMS IN MARCHANTIA DOMINGENSIS

EMMA N. ANDERSEN

(WITH NINETEEN FIGURES)

Introduction

The different statements occurring in textbooks and in research papers, concerning the discharge of antheridia, made it seem desirable to study the antheridia of *Marchantia*, to find if possible the mechanism which discharges the antherozoids.

THURET (17) in 1856 observed the forcible discharge of sperms in *Conocephalus conicus*. PEIRCE (15), working later, stated that the mechanism effecting expulsion of the sperms in *Asterella californica* consists of two parts: (1) the water-absorbing matrix consisting of gelatinized mother cells with thin walls, in which the sperms lie, which distend the mature antheridium still inclosed by the single layer of peripheral cells; (2) the thin walled, large celled, water-absorbing tissue which forms the walls of the chambers in which antheridia develop. These two, the tissue and the water-absorbing matrix, expand in opposite directions as they absorb water, the former tending to decrease and the latter to increase the size of the chambers containing antheridia. The two pressures would tend to offset each other and affect nothing if they met on all sides of the antheridia. The chambers in which the antheridia lie are open above, and are covered by the chlorophyll-containing, smaller celled, and more rigid tissue. The antheridia are distended in all directions and are compressed on all sides except from above. PEIRCE concluded that the distending and compressing strains finally result, under these considerations, in the rupture of the antheridium and the explosive discharge of its contents through the mouth of the chamber.

CAVERS (3), working on the same genera, observed that explosive discharges took place on warm, sunny days, and that they were especially frequent when the plants were exposed to direct sunlight; they were not observed on dull days, nor when the plants were shaded. He accounts for the pressure resulting in the expulsion of

the antherozoids as caused by water absorbed by various mucilage cells; and if any antheridia are ripe, the walls of the antheridium as well as of the sperm mother cells, which at this time are largely mucilaginous, also take up water and become swollen. KING (9), who also worked on *Conocephalus* stated that moisture alone may be the inciting cause of the explosion; his statement was based on observations made in the afternoon after spraying.

CAMPBELL (1), in his treatment of antheridia, says that the mature walls of the sperm cells become mucilaginous; and when the mature antheridium is wet, the swelling of this mucilaginous mass bursts open the antheridium and sets free the sperm cells, from which the spermatozooids are liberated by complete dissolution of the cell wall. STRASBURGER (16) states that the spermatozoid mother cells are ejected from the antheridium.

GOEBEL (7) observed in the Marchantiaceae a great increase of radial diameter of the cells of the wall of the deeply sunken mature antheridia. Here the expansive pressure was against the wall of the cavity in which antheridia are located.

The opening of the cavity in many forms is drawn into a point projecting over the upper layer. GOEBEL thinks this serves the same purpose as the point of a sprayer; the antheridial wall acts as the wall of the rubber bulb when squeezed. In many liverworts the ejection of spermatozooids occurs as if from this apparatus.

Discharge of the antheridia of *Marchantia domingensis* in free-hand sections of the antheridial branches was observed under the low power of the microscope. The great inward distention of the antheridial wall cells, with the exception of the cells at the apex and the highly colored cells of the disk lining the antheridial chamber, was very striking. The cause of this distention was sought; and a brief study was made of the development of the cells of the antheridial wall, the chemical nature of the cell walls and cell contents of the antheridia, and the antheridial branches, in an effort to learn more about the mechanism of the discharge of the antherozoids.

Materials and methods

Plants of *Marchantia domingensis* were grown for a number of years in flats in the greenhouse at a temperature of approximately

22 C., and watered daily. The thalli grow luxuriantly, and at first produce only cupules (fig. 1). A very few antheridia can also be seen. Later abundant antheridial branches are produced, and when these reach the size shown in fig. 2, the antheridia near the center of the disk are discharging the antherozoids. The work was done on antheridial branches of this age. Later, when the antheridia have discharged the antherozoids, the antheridial disks are much greater in diameter and are more deeply lobed. In fig. 3 they are shown with archegonial branches. The old antheridia lie close to the thalli, and their disks have a greater diameter than the archegonial disks.

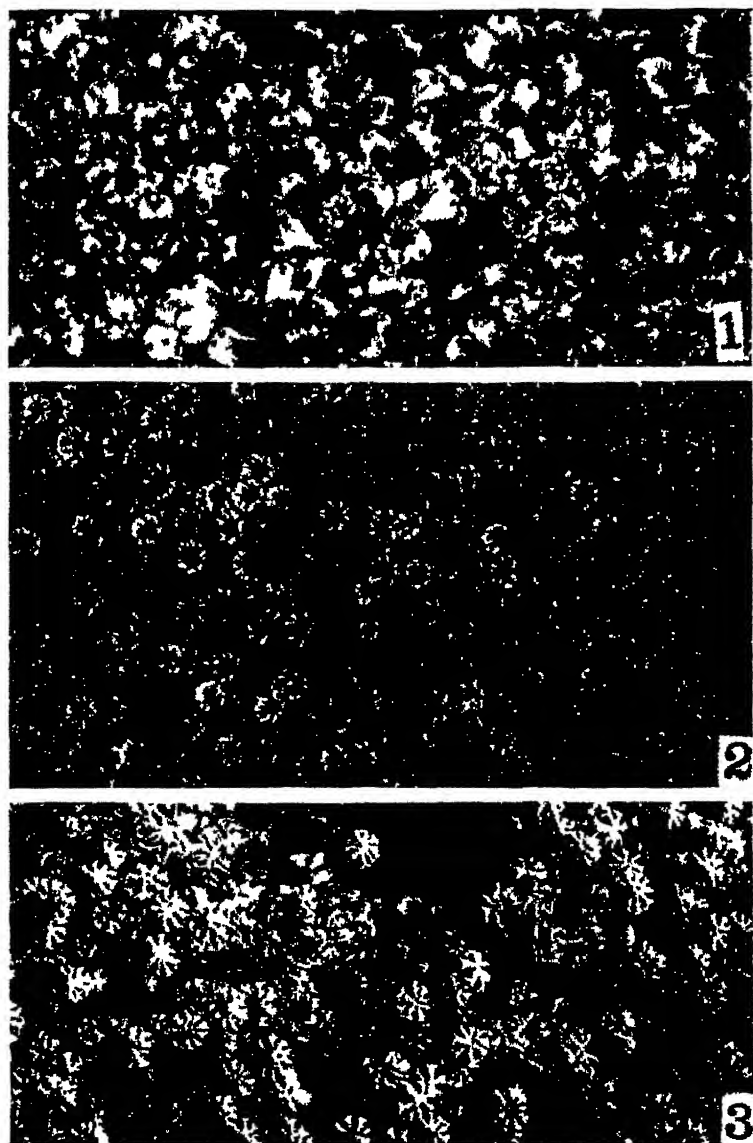
Freehand sections of living material, made with a razor and freezing microtome sections, were used in the microchemical study. A brief outline of each microchemical test is given where the substance investigated is discussed in this paper. Details of tests when not given may be found in ECKERSON (4), TUNMANN (18), MOLISCH (12), and MANGHAM (11). The tests as outlined by ECKERSON are used for the most part.

Material was fixed in the following solutions: acetic alcohol, Carnoy's, chromo-acetic, Flemming's, formalin alcohol, formalin acetic alcohol, and platinum chloride. The material was dehydrated in the usual manner up to 70 per cent alcohol. Free-hand, freezing microtome and paraffin sections were made from material in 70 per cent alcohol in the study of the development of the cells of the antheridial wall. Material fixed in Flemming's, Carnoy's, platinum chloride, and formalin acetic alcohol when sectioned proved to be satisfactory. Photomicrographs¹ were taken of free-hand sections fixed in Flemming's stronger solution, sectioned from 70 per cent alcohol and mounted in glycerin on the slide (figs. 4-9).

Investigation

The antheridial branches of *M. domingensis*, as described for the genus *Marchantia* (2, 5, 16), consist of a lobed, horizontal disk and erect stalk. The disklike thallus is dorsiventral, and consists of three regions: upper epidermal, chlorophyll bearing, and lower compact part (fig. 5). The chlorophyll region and the turgid cells

¹ The writer wishes to express her appreciation to Dr. ELDA R. WALKER for aid in photomicrographic work, to Dr. SOPHIA H. ECKERSON for suggestions, and to Professor T. J. FITZPATRICK for reading manuscript and proof.



FIGS. 1-3 —Fig. 1, thallus showing cupules and very few antheridia; fig. 2, antheridial branches and cupules; fig. 3, old antheridial branches and archegonial branches; $\times 1$.



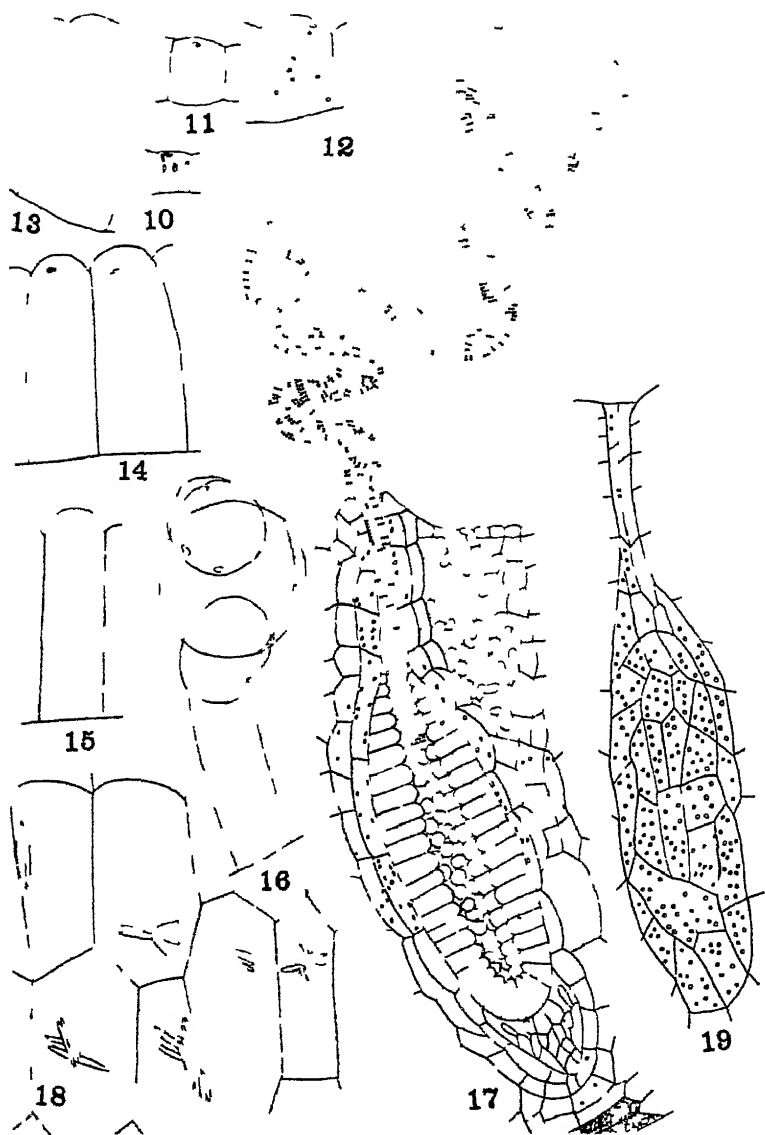
FIGS. 4-9.—Photomicrographs of mostly immature antheridia (dark area in center is spermatogenous mass): Fig. 4, immature antheridium at left, antheridium about to discharge at right, $\times 45$. Figs. 5, 7, 8, photosynthetic chambers and turgid cells lining antheridial cavity, figs. 5, 8, $\times 45$; fig. 7, $\times 100$. Fig. 6, different stages of development; antheridium at extreme left has discharged contents and collapsed, $\times 40$. Fig. 9, one to left showing transparent condition of wall cells at discharge; $\times 85$.

lining the antheridial cavity are shown in fig. 7. Scales and rhizoids occur on the ventral surface of the disk. The antheridia arise singly in flasklike depressions which extend down into the compact tissue. The cavities or depressions are surrounded by air chambers containing branched rows of photosynthetic cells and epidermal pores. Antheridia do not all mature at once, the oldest being near the center. The antheridia are stalked (figs. 4, 5, 8), and contain a mass of spermatogenous cells inclosed by a wall consisting of a single layer of sterile cells. In figs. 4-9 the spermatogenous mass appears black, and all antheridia show walls whose cells have begun to elongate inwardly, except the antheridium on the left in fig. 4. In the cavities surrounding the stalk are turgid cells or paraphyses.

In free-hand sections of antheridial branches of *Marchantia domingensis* and *M. polymorpha*, it is possible to obtain them sufficiently thin to enable one to see antheridia in their chambers. It was observed under low power, that many of the mature antheridia soon after being placed in water on the slide began to discharge their contents, which consisted of sperm mother cells containing sperms or antherozoids instead of free sperms. The biciliated sperms or antherozoids rotated rapidly in the mother cells as they were ejected, but the sperms were not free to swim and escape from the mother cell while in the antheridium. Although many antheridia were injured in mounting and sectioning, there were always a sufficient number of uninjured ones apparently behaving normally. When conditions were optimum the sperm mother cells were ejected in a relatively short time in smokelike columns.

In young antheridia of *M. domingensis*, the cells of the wall are more or less cubical, and contain a nucleus and a number of chloroplasts lying in the cytoplasm (fig. 10).

When the antheridia have reached an intermediate stage in growth and an almost full-grown but not mature stage, the cells of the antheridial wall have become elongated, and apparently many chloroplasts have begun to disintegrate (figs. 11, 12). After disintegration granules are present (fig. 13), which become blue when iodine is added, indicating the presence of much starch. At the time of dispersal, the cells of the antheridial wall contain most frequently only granules imbedded in the cytoplasm (fig. 13). Occasionally at



FIGS. 10-19.—FIGS. 10-16 development of cells of antherial wall $\times 450$. Fig. 17, antheridium displaying strobiloid column of sperm mother cells, $\times 120$. Fig. 18 probably maltotriose crystals in tissue below antheridia, $\times 450$. Fig. 19 pigmented cell lining antherial chamber $\times 120$.

dispersal some chloroplasts are still intact, as shown in the upper part of the antheridium in fig 17 and the uppermost droplet in fig 16. Paraffin sections of antheridia whose walls are distended inwardly often show a nucleus (fig 14). Distended cells of the wall which contain no nuclei occasionally are found in paraffin sections (fig 15) but nuclei were never seen in living discharging antheridia (figs 16, 17).

At the stage when the antheridia are fully mature and eject their sperm mother cells when placed in water the cells of the wall are greatly distended with the exception of those at the apex (figs 5-9). The cells of the wall distend to such an extent that they ultimately almost fill the spermatogenous cavity. They contain fewer granules indicating that little starch is present. The antheridia are kept from receding in the chamber by a number of turgid paraphyses surrounding the antheridial stalk at the base of the chamber (fig 17).

The sperm mother cells have also increased in size. They appear to be pushed out slowly in smokelike columns by the greatly distended wall cells which have elongated inwardly, making the spermatogenous cavity smaller. The cells lining the entire antheridial chamber, or usually more than the upper half, are now highly colored, red predominating (fig 19). They turn a bright red when treated with acid, and when treated with dilute alkali they become blue, indicating the presence of anthocyanin. Freezing microtome sections of the cells lining the antheridial chamber show that the walls are colored uniformly, the corners usually appearing more deeply colored. The cells contain many chloroplasts. Attempts were made to determine whether or not the cell sap of these cells also contained anthocyanin, but the results were always negative. NAGAI (13), however, states that not only the walls of *M. polymorpha* but also the cell sap contained anthocyanin. The change in color of the cells lining the antheridial chamber no doubt bears a significant relation to the chemical nature of the cells of the antheridial wall, as well as to their contents and to the ultimate discharge of the spermatogenous mass. ONSLOW (14) states that one idea is that red pigment assists the diastase activity by screening it from deleterious rays, thus facilitating the hydrolysis of starch and subsequent translocation. Those antheridia of *M. domingensis* representing an inter-

mediate stage of growth contain relatively large amounts of starch in the cells of the antheridial wall, while the mature antheridia, screened by the reddish cells of the wall of the chamber, contain relatively small amounts. It may be that the decreased starch content in mature antheridia is due to the influence of the anthocyanin cells lining the chamber; on the other hand, the pigmented cells may prove to be merely a mechanical device to aid inward distention of cell walls of the antheridial wall and the subsequent discharge of sperm mother cells. That there is a greater need for a stronger upper lining of the antheridial chamber than the lower, is because the upper part is adjacent to the air chambers containing branched rows of photosynthetic cells, thus giving little support to the antheridial lining (figs. 5, 7, 17).

As soon as most of the spermatogenous material has been expelled, hyaline drops become apparent. The writer first thought that these drops were plastids greatly enlarged, since chloroplasts in ruptured cells of the disk have been observed to be much swollen when water enters the cells. ZIRKLE (20) believes that the swelling of chloroplasts of fern prothallia when water enters a cell is due to imbibition of water by the contents of the central vacuole of the chloroplast, which remains separated from the outer water by a surface film. Upon further observations, the writer noted that the apices of the cells of the antheridial wall of *M. domingensis* near the center of the antheridium appear to dissolve. As these walls dissolve the cell contents escape in one or more spheres or films. In the process of exit from the cells (fig. 16) these drops are more or less compressed, but upon being freed they assume a spherical form. These droplets are ephemeral and sooner or later burst. Emerging droplets do not constitute a rare phenomenon, since many workers have observed films about droplets of protoplasm which have emerged from cells (8). The droplets probably consist of cytoplasm, broken down chloroplasts, and dissolved wall material. Each droplet appears to contain a large vacuole. It was often observed that the greater portion of the granular material is attached to one side of the droplet, like the granular stroma of swollen chloroplasts. Very rarely was a drop seen in which the plastids were still intact. This condition is shown in the uppermost drop (fig. 16). The droplets are exuded into

the spermatogenous cavity and at this time are much distended. They appear to aid in expelling the few remaining sperm mother cells. GARDINER and ITO (6), investigating the structure of the mucilage secreting cells of *Blechnum occidentale* and *Osmunda regalis*, report mucilaginous hyaline drops and droplets. Each drop had its own protoplasmic reticulum and contained tannin. The spermatogenous cells of *M. domingensis* indicate a slight trace of tannin, but none was detected in the cells of the antheridial wall.

Microchemical tests and results

MEMBRANES

MUCILAGE.—According to KRAEMER (10), cellulose walls may be divided into the following groups: ligno-cellulose walls, protective cellulose walls, reserve cellulose walls, mucilage cellulose, and mineral cellulose walls. He speaks of mucilage cellulose walls consisting of cellulose and mucilage as found in all parts of the plant, and states that they dissolve or swell in water and are colored blue or yellowish with iodine. They are stained also with alcoholic or glycerin solutions of methylene blue. The antheridia of *Marchantia domingensis* were treated with iodine, but the cell walls of the distended antheridial wall cells were colored neither blue nor yellowish unless treated for several hours. The spermatogenous mass became very yellow. Each mother cell wall, however, had only a slight trace of color if any.

YOUNGKEN (19) also states that mucilage may be demonstrated in plant tissues by placing sections in a deep blue solution of methylene blue in equal parts of alcohol, glycerin, and water on a glass slide, allowing them to remain in the solution several minutes, and then draining and mounting them in glycerin. The cells containing mucilage should exhibit bluish contents. The results from this method indicated the presence of mucilage in the cell walls of the antheridia. The cell walls of the antheridial branch are colored blue. Chlorzinc iodide colors the cell walls of the antheridial branch and young antheridial walls. Usually, however, a thick inner layer which stains less deeply is formed (fig. 11) in the antheridial wall cells. Later, the part of the layer that extends from the center to the apex deepens in color (fig. 13). It is probably mucilage.

The following tests for mucilage, used by GARDINER and ITO (6),

were also used. They found that alcohol caused abnormal shrinking of the mucilage and was attended by great distortion of structure. While alcohol always seemed to arrest activity of the cells of the antheridial wall of *M. domingensis*, they were never greatly distorted. Saturated watery picric acid produced great swelling of the mucilaginous contents, which finally ended in bursting the cell and permitting the mucilage to escape. While sections of the antheridial branches were used, attention was again focused on the cell walls of the antheridial wall with negative results. With iodine, mucilage stained a clear yellow. The antheridial branches of *M. domingensis* were sectioned and used, and only the spermatogenous mass appeared yellow. A 2 per cent chromic acid solution was used on some sections and a 1 per cent osmic acid solution on other sections of antheridial branches. Since but a trace of tannin had been obtained in the sections, these tests were also negative.

HEMICELLULOSE.—Sections of antheridial branches were heated in 3 per cent sulphuric acid over a water bath for 2 hours. The walls neither completely nor partially hydrolyzed, indicating that hemicellulose was not present. Sections of the antheridial branch were treated with warm water, alcohol, ether, and then heated with an excess of concentrated nitric acid and placed in a moist chamber. Mucic acid crystals, indicating the presence of galactan, could not be detected.

Sections of antheridial branches were placed on a slide in a 4 per cent orcin solution, after which a drop of concentrated hydrochloric acid was added. They were then heated to the boiling point. After boiling, the antheridia were usually a structureless mass. The blue or violet color apparently occurs in the cell walls of the spermatogenous mass, and sometimes it seems to appear in cells of the antheridial wall, in cell walls at the edge of the disk and stalk, and in the portion of the disk under the antheridial chambers. A blue or purple color indicates the presence of pentoses. Sections of the male branch were placed on slides and treated with a 1 per cent solution of phloroglucin, after which a drop of concentrated hydrochloric acid was added. They were then heated for ten minutes. As in the former case, the antheridia became structureless. The same walls were colored red, again indicating the presence of pentoses.

CELLULOSE.—Sections of antheridial branches were treated with

75 per cent sulphuric acid and iodine potassium iodide. The cell walls of the cells of the epidermal and chlorenchymatous tissue in the upper part of the disks, of the regions between and below antheridia, of the scales and rhizoids, and of the stalk of the antheridial branch (with the exception sometimes of a few small interrupted areas along the edge of the disk) were colored blue, which indicates the presence of cellulose. The cell walls of the immature antheridial wall were blue, but only a trace was found in sperm mother cell walls. Sections were also observed with polarized light, and the same walls were doubly refractive. Chlorzinc iodide was also used. It confirmed the previous test and in addition colored the epidermal cells of the antheridial branches and outer tangential cell walls of the antheridial wall yellow, indicating the presence of cutin.

PECTIC SUBSTANCES.—Sections of the male branch were placed on a slide in a dilute solution of ruthenium red. Excluding the antheridia, the cell walls of the remaining cells of the disk and stalk of the antheridial branch stained red, which indicates the presence of pectic substances. Sections of the male branch were heated in 2-15 per cent hydrochloric acid over a water bath for 0.5-2.5 hours. At the end of that time, after a thorough washing, the cell walls of the antheridial branch excluding the antheridia stained as well as at the initial staining, with the exception of the rhizoids and some scales. Some of the scales dissolved completely, indicating that they consisted entirely of pectose.

Sections of the antheridial branch were heated in 2 per cent ammonia for one hour. They stained as well as at the initial staining, indicating again that the walls are not pectic acid but pectose or calcium pectate, and must first be treated with a weak solution of hydrochloric acid. Sections of the antheridial branch were heated in 2-15 per cent hydrochloric acid from 15 minutes to one hour, thoroughly washed, and then heated in 5 per cent ammonium oxalate for 30 minutes to one hour. The cell walls of the antheridial branch (excepting antheridia) no longer stained, indicating that the walls contain calcium pectate.

STORAGE SUBSTANCES

STARCH.—Iodine potassium iodide solution was used. The cells of the chlorenchymatous portion of the disk and of the regions between

and under the antheridia, the anthocyanin cells lining the cavity of the antheridia, and the cells of the upper part of the stalk of the antheridial branch usually contain abundant starch. Disks occur, however, in which little or no starch is found except in antheridia, or only in the chlorenchymatous portion with a little in antheridia. Other disks occur where starch is found largely in the tissue between and under the antheridia, together with a little in a few of the antheridia. Again other disks may be found in which starch occurs only in the layer immediately under the antheridia, with some in a few of the antheridia. Starch when it occurs in antheridia is always found in the cells of the antheridial wall. The youngest antheridia near the periphery of the disk rarely if ever contain starch, and the discharging ones usually also contain very little. The cells of the walls of the remaining antheridia, representing the intermediate stages, contain starch in varying amounts. Of these, the antheridia nearest the maturing ones contain the least.

SUGARS.—Antheridia of all ages were treated with 15–20 per cent sodium hydroxide and copper tartrate, and then heated (Flückiger's test). No reduction was obtained on gently warming or without heating, indicating that probably fructose was not present. Upon longer heating crystals of cuprous oxide were observed, however, which may indicate that glucose was present. Crystals were also observed in the cells above and below the antheridia, when branches were tested.

The phenylhydrazine hydrochloride and sodium acetate test was also used. On some slides antheridia alone were placed in the solution, and on others sections of the antheridial disk as well as antheridia were used. Some sections were heated, others were kept at room temperature for two days, and neither fructosazone nor well formed glucosazone crystals were obtained in the cells with or without heating.

Sections which gave no crystals after two days at room temperature were heated on a water bath for about 40 minutes, to see whether crystals would form. None formed, thus indicating the absence of sucrose.

Sections were heated 15, 35, 45, 60, and 75 minutes respectively, and examined every few days over a period of four months (11).

Sections which had been heated 45 minutes on a boiling water bath gave good crystals in about one month. More crystals had formed in two months. At the end of three months many sections that had been heated one hour in the reagent now also contained good maltosazone crystals (fig. 18). At the end of four months no increase in any region could be ascertained. Many of the immature antheridia which had not yet elongated their cell walls were covered with crystals, probably indicating diffusion of sugar through the walls; but the mature antheridia which had discharged their contents contained very rarely more than one cluster of crystals. In many of the disks, the cells constituting the layer beneath the antheridia, as well as the cells between the antheridial chambers, contained maltosazone crystals. Similar large clusters of crystals commonly occur on the exterior portion of the disk. As in the case of the young antheridia, I have interpreted the crystals as having been formed from sugar diffused from the chlorenchymatous tissue. Since the cell walls of the wall cells and mother cells of the antheridia just before discharge consist largely of a substance which gives the pentosan reaction, and the cells of the antheridial wall contain little glucose if any, and also a small amount of maltose, it would seem that if osmosis indeed enters into the mechanism of spermatogenous discharge, it plays but a minor rôle.

MINERAL SUBSTANCES

Sections of antheridial disk were treated with 1 per cent benzidine in 3 per cent hydrochloric acid, and many needle crystals of benzidine sulphate were obtained. The storage region of the disk located below the antheridial chambers contained abundant crystals of sulphate. Antheridia and chlorenchymatous tissue as well as scales and rhizoids gave negative results.

In treating sections of antheridial disk with a saturated solution of ammonium chloride in water, with a few drops of ammonia and sodium phosphate crystals to make a 0.1 per cent solution, a few ammonium-magnesium-phosphate crystals appeared in the antheridia. The cells of the antheridial wall coalesce completely upon warming, but in spite of this fact the magnesium crystals are primarily found only in the peripheral cells of the antheridium. The significance of magnesium in the cells of the antheridial wall is not clear.

It may have been derived from the broken-down chloroplasts, and its presence may change the viscosity of the protoplasm, slightly shortening the existence of extruded drops. Other mineral tests gave negative results.

Discussion

In viewing the discharge of *Marchantia domingensis*, the great inward distention of the cells composing the antheridial wall, with the exception of the apical cells is the striking feature. Since this distention might be due to osmotic pressure, sugar tests of the cells of the walls were made. All the tests used were microchemical, in order to obtain localization. While there were immature antheridia which had not yet elongated their walls, and which were covered with crystals, the antheridia which had discharged their contents contained, if any, rarely more than one cluster of maltosazone crystals. The pentose reaction was obtained in the cells of the sperm mother cells and sometimes in the antheridial wall, but the reaction for mucilage occurred in both. The behavior of the antheridial wall, however, was that of a pentosan in having the power of swelling and of taking up a large quantity of water. It is also mucilaginous in character like the pentosans. The cell walls of the young antheridial wall also gave the cellulose reaction, and the exterior or surface walls were covered besides with a thin layer of cutin. The theory that osmotic pressure was the cause of the great inward distention was abandoned when the cells of the antheridial wall indicated that they were principally mucilage or pentosan, and contained little sugar.

While discharging antheridia contained little starch, those in intermediate stages often contained a great amount; of the immature antheridia, those nearest maturity usually contained the least. Since the cells lining the antheridial chambers become colored at approaching maturity, red predominating, it was thought that those cells might influence chemical activities occurring in the cells of the antheridial wall. If they increase the temperature of the cells of the antheridial wall, there might be increased pentosan formation. This red pigment or anthocyanin, found in the cells lining the antheridial cavities, might also facilitate the hydrolysis of starch, and thus account for the decreased amount of starch usually found in antheridia that are discharging their contents. While it seems probable

that the pigmented cell walls of the antheridial chamber may affect these chemical activities, on the other hand it may prove to be a mere mechanical device for aiding sperm mother cell discharge. The

TABLE I
SUMMARY OF MICROCHEMISTRY OF CELLS OF ANTHERIDIAL BRANCH
OF *MARCHANTIA DOMINGENSIS*

MEMBRANES	
MUCILAGE	PENTOSANS
Cell walls of antheridial wall and sperm mother cells	Cell walls of spermatogenous cells and sometimes antheridial wall cells Cell walls at edge of branch Cell walls of ventral region of disk
CELLULOSE	PECTIC SUBSTANCES
Cell walls of immature antheridial wall cells All cell walls of antheridial branch with trace in spermatogenous wall cells and with exception sometimes of a few small interrupted areas along edge of disk	All cell walls of stalk and disk of antheridial branch with exception of antheridia
CUTIN	PECTOSE
Epidermal cells of antheridial branch Outer tangential cell walls of antheridial wall	Cell walls of scales and rhizoids
CALCIUM PECTATE	
	All cell walls of stalk and disk of antheridial branch with exception of antheridia, scales, and rhizoids
STORAGE SUBSTANCES	
STARCH	SUGARS
In plastids of cells of photosynthetic cells in air chambers In plastids of anthocyanin cells and cells between and under antheridia In plastids of cells in upper part of stalk In plastids of antheridial wall cells	GLUCOSE Cells of antheridial wall Cells between and below antheridia MALTOSE Cells of antheridial walls Cells between and below antheridia Cells of epidermal area
MINERAL SUBSTANCES	
SULPHATE	MAGNESIUM
Cells of the ventral region of disk	Cells of the antheridial wall

most important single factor occurring in the spermatogenous discharge seems to be the enormous inward distention of the cells of the antheridial wall, although several other factors aid in the discharge.

The turgidity of the cells lining the cavity causes the cells of the

antheridial wall to distend toward the interior of the cavity, bringing considerable pressure to bear on the spermatogenous mass. At the same time the sperm mother cell walls are also increasing in size, resulting in their expulsion in smokelike columns. Basal cells or paraphyses surround the stalk of the antheridia, and prevent them from receding into the cavity. They aid in maintaining the contact between the apex of the antheridium and the opening that leads to the surface of the disk, thus facilitating discharge.

Finally, in the cells of the antheridial walls a katabolic process is taking place, which results in the formation of hyaline drops, probably composed of cytoplasm, broken-down plastids, and some of the cell wall substance. These hyaline drops, usually ephemeral, are discharged into the spermatogenous cavity; and as they fill the cavity, which by this time is much diminished in size, they seem to aid in expelling the few remaining sperm mother cells. The mass of spermatogenous material ejected consists of sperm mother cells which contain the sperms. The sperms can be seen rotating rapidly for some time in each cell. Eventually complete dissolution of the cell takes place and the sperms are free to swim.

Table I summarizes the microchemistry of the cells of the antheridial branch.

Summary

1. In *Marchantia domingensis*, the cell walls of the cells of the antheridial wall and of the sperm mother cells, before discharge consist largely of mucilaginous material which gives the pentosan reaction in the sperm mother cells, and sometimes in the antheridial wall cells. The cell walls of the latter when immature also give a cellulose reaction, and the exterior or surface walls are covered with a thin layer of cutin.

2. Excluding the antheridia, almost all of the cell walls of the disk and stalk of the antheridial branch, as well as the scales and rhizoids, give both a cellulose and a pectic reaction.

3. Sugar reactions in the antheridial peripheral cells or wall cells by means of Flückiger's test are questionable. Glucose may be present.

4. The greatest number of maltosazone crystals is found on the exterior region of non-mature antheridia. Maltosazone crystals are

obtained in many cells of the disk between the antheridia, and also in cells occupying the region below them.

5. The cell walls of the cells lining the entire or a little more than the upper half of the antheridial cavity contain anthocyanin at maturity, which may tend to increase diastase activity, katabolic activity, and incidentally also pentosan formation; on the other hand the pigmented lining may function wholly in a mechanical capacity.

6. The significance of magnesium in the cells of the antheridial wall, as well as the abundance of sulphate in the ventral region or in the layer of cells beneath the antheridia, is not clear. The magnesium may change the viscosity of protoplasm.

7. Discharge of the spermatogenous mass depends upon: (1) the inward distention of the cells of the antheridial wall caused by the large amount of water imbibed; (2) the simultaneous swelling of the spermatogenous cell walls; (3) the pressure exerted by the turgid more or less pigmented cells lining the antheridial cavity and causing its gradual diminution as the spermatogenous mass is being ejected in smokelike columns; (4) the tendency of the swollen basal cells or paraphyses in the antheridial chamber to keep the antheridium in its upright position; (5) the force emitted by expelled hyaline drops probably composed of cytoplasm, broken down plastids, and wall substance.

8. In the ejection of spermatogenous material the cells of the antheridial wall play a major part.

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[Accepted for publication July 25, 1930; delayed through author's revision]

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MARCESCENT LEAVES OF CERTAIN SPECIES OF QUERCUS

EARL E. BERKLEY

(WITH ELEVEN FIGURES)

The abscission of leaves was first studied by VON MOHL (5); since then considerable work has been done, although little attention has been given to the phenomenon of marcescent leaves. TISON (4) observed both marcescent and deciduous leaves in *Carpinus betulus*, *Fagus sylvatica*, *Quercus hispanica*, and *Q. pedunculata*. In the deciduous leaves the abscission layer was formed in the autumn, followed by the ligno-suberization of the other cells of the scar, giving rise to a protective layer. In the petioles of the marcescent leaves, only partially formed abscission layers were found, marked by the breaking apart of small groups of cells as a result of gelatinization of their walls. For the most part the marcescent leaves were situated on the short lateral branches, and it was thought that the marcescent condition was induced by injury due to frost or freezing, which suddenly killed the petiole and thus prevented further development of the abscission layer. The marcescent leaves were abscised the following spring by the development of an abscission layer just beneath the ligno-suberized area. A similar meristematic region was formed just beneath the protective layer of the stubs left after the leaves had fallen. LEE (1) confirmed TISON's work on *Carpinus*, but he did not investigate the marcescent leaves of *Quercus*.

LLOYD (2, 3) observed marcescent leaves on a number of forest trees. He did not examine petioles of all species observed, but found partially formed abscission layers in some of the species examined. In further work on cotton, he observed that in certain varieties the bolls died and withered on the stem without falling.

In the late autumn of 1927, the fact that the leaves of a certain oak tree did not fall attracted the attention of the writer. Observations were continued throughout the ensuing winter, and during the period extending from May, 1928, to May, 1929. Collections of

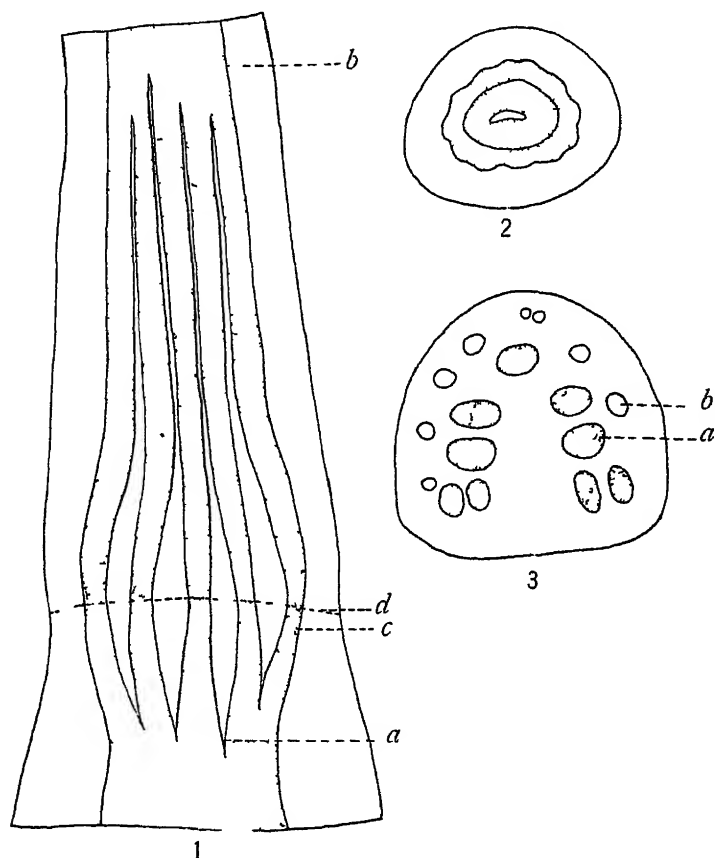
petioles from various species of *Quercus* were taken at frequent intervals. These were preserved in formalin acetic alcohol, and later sectioned according to the paraffin method. Petioles collected during the winter of 1929 were demineralized by treating with 25-75 per cent hydrofluoric acid for a period of 2-4 weeks.

The petioles of the various species of *Quercus* studied are very similar, varying only in minor details. Most of them are rather slender and almost cylindrical. Near the base the petiole becomes somewhat larger, and there is a slight constriction at the point of its junction with the twig. Three or more leaf traces join the vascular cylinder in the leaf gap, but in the base of the petiole these divide and diverge to form a number of separate strands surrounded by pith and cortical cells (figs. 1a, 3a). Above the abscission zone these separate strands are again united (fig. 1b), forming a cylinder similar to that of the stem (fig. 2).

As the separate bundles diverge, the mechanical tissue surrounding the vessels gradually decreases, until finally within the abscission zone it is practically lacking (fig. 1c). On the distal side of the abscission zone the mechanical tissue gradually increases, until a complete cylinder is formed. On the proximal side there is an increasing development of sclereids (fig. 3b), extending to the union of the petiole strands with the vascular cylinder of the stem. The sclereids are most numerous on the upper side, where in some cases they form an almost continuous sclerenchyma in the axil of the petiole strands, at which place they join the main cylinder. Crystals of calcium oxalate are abundantly distributed throughout the cells of the petiole (figs. 4-6), but they do not occur in the abscission layer during the process of abscission (figs. 4, 5, 7).

In *Quercus*, the first processes initiating development of an abscission layer take place shortly before the fall of the leaf. The first noticeable change is an increase in the density of the protoplasm in the cells of the abscission zone, and an accumulation of food in these cells. Subsequent enlargement and division of cells proceed more or less irregularly (figs. 5, 7). The distal cells of the zone elongate, and their walls swell and become distorted, in some cases being completely digested (fig. 4a). The inner cells of the cortex on the lower side of the petiole are the first to be affected. From this point develop-

ment extends upward, around the bundles, and outward toward the epidermis. The epidermis on the upper side is reached last, and in

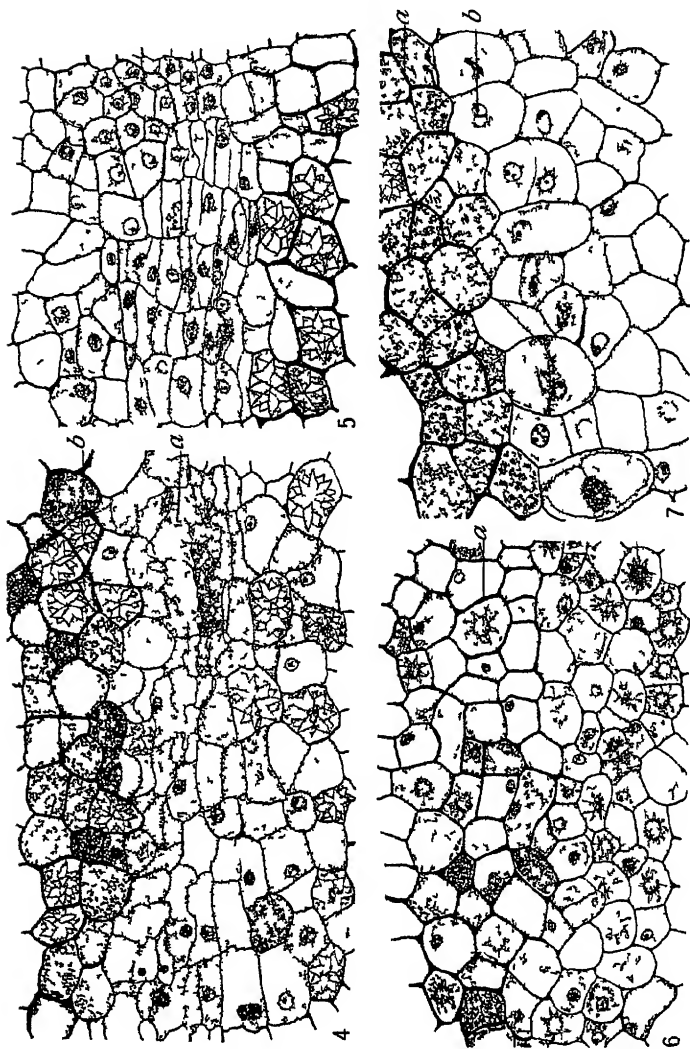


FIGS 1-3—Fig 1, longitudinal diagram illustrating details of petiole in region of abscission, *a*, fibrovascular cylinder separating into separate bundles proximal to abscission zone, *b*, fibrovascular cylinder after separate bundles have reunited above abscission zone, *c*, reduction of mechanical tissue, *d*, line of abscission. Fig 2, cross-section near point 1b. Fig 3, cross section proximal to abscission zone, *a*, separate bundle, *b*, sclerenchyma.

many instances the tissue here is severed by mechanical force before the cell walls have been digested.

Apparently abscission may be effected in two ways, either by cell division and digestion of the cell walls, or by digestion of the cell

walls alone. When abscission is accompanied by cell division, there is some cell division in the mechanical tissue also (figs 5, 8*a*), but



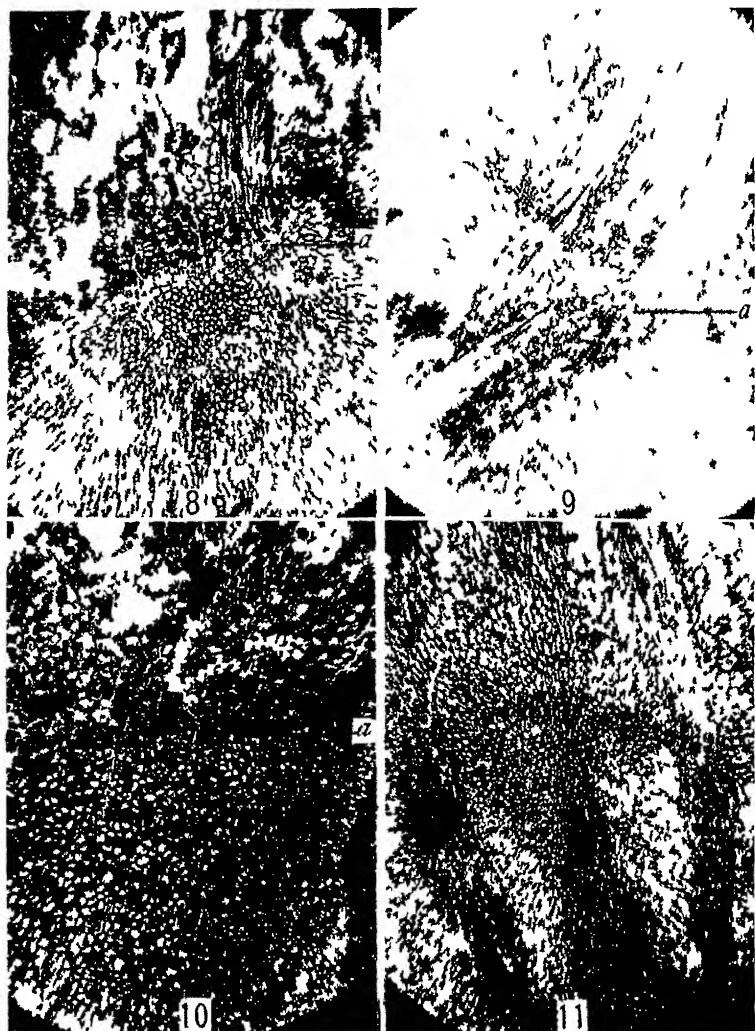
FIGS 4-7.—Fig 4, section of petiole through abscission layer, *a*, breaking apart of cells, *b*, aggregates of calcium oxalate crystals. Fig 5, section of petiole showing abscission region in normally deciduous leaf. Fig 6, section of petiole before cell division takes place in spring, *a*, ligno suberized area characterized by heavier cell walls. Fig 7, section of petiole of marcescent leaf showing division of cells initiating abscission layer, *a*, cells ligno suberized during previous autumn, *b*, cells in process of division.

the new cells are much smaller than those adjacent. When abscission is not accompanied by cell division, the cell walls of the mechanical and other tissue are digested. Separation usually takes place

in the region of the middle lamella, and breaking of the petiole takes place in an abscission plane. Often the fibrovascular bundles do not break in the same plane with the cells of the other tissue, as the plane of abscission may form parallel with the bundles for the distance of a few cells before it cuts across the bundle itself.

Abscission in the various species of *Quercus* studied is often accompanied by irregular cell division. The abscission layer is developed in the more distal cells of the abscission zone, and those on the proximal side become ligno-suberized to form the protective layer over the scar. As previously stated, the cell walls in the abscission layer are digested precisely as they are in those petioles in which abscission takes place unaccompanied by a division of cells. When cell division occurs, subsequent enlargement of the new cells formed produces elongation of the petiole in this region. No divisions, therefore, occur in the vessels which, unable to elongate to conform to the increased petiole length, are subjected to stress. This stress, together with digestion of the walls of the vessels, finally produces a rupture and pulls them apart (fig. 9a), often before the petiole is severed from the stem. If there is no cell division, the walls of the vessels and the cell walls of the mechanical tissue are digested and broken apart simultaneously with the cell walls of the pith and cortex. The cuticle and sometimes the outer walls of the epidermal cells are the only parts that do not show alteration in the plane of the abscission layer.

Before the leaf blade is entirely dead, a greenish band, in the form of a groove, appears at the base of the petiole, in the region where a little later the line of cleavage separates the leaf from the twig. This shallow groove, extending around the petiole, apparently results from the failure of the actively dividing cells in the region of the developing abscission layer to enlarge as rapidly as do those cells lying immediately adjacent on either side. The line of cleavage usually cuts through the distal border of the groove, but the groove does not necessarily indicate the precise location of the abscission layer. In the deciduous leaves of *Quercus*, in most cases at least, the plane of cells delimited peripherally by this groove becomes ligno-suberized, either before or after leaf shedding, to form the pro-



FIGS 8-11—Fig 8, photomicrograph of longitudinal section showing abscission region just before leaf fall, *a*, abscission layer through mechanical tissue Fig 9, abscission in normally deciduous leaf, *a*, rupturing or pulling apart of spiral vessels Fig 10, first cell division in formation of abscission layer in petiole, *a*, tyloses in cells of ligno suberized tissue Fig 11, line of demarcation between living tissue of stem and dead ligno suberized tissue of petiole of marcescent leaf (in which there has been no development of abscission layer)

protective layer seen in the leaf scar. This region of ligno-suberization involves 10-18 layers of cells. Tyloses form during the process of abscission, or immediately afterward in the cells of the protective layer (fig. 10a), these can be noticed first in the cortical cells, and finally in the vessels themselves. Such tyloses often appear before the vessels are severed.

Generally the groove is less pronounced in those leaves which die and wither on the stem-marcescent leaves, and usually they lose their green color until growth activity begins in the spring, at which time the dead leaves are abscised. In such marcescent leaves the petiole has withered and the cells on the distal side of the plane extending through the groove are dead. In this condition the groove, if previously formed, has become so completely blended with the shrunken petiole as to be inconspicuous. The layer of cells extending across the petiole in the plane of this groove has become ligno-suberized in the same manner as those of the leaf scar already mentioned (figs. 6a, 11). In early spring a meristematic region develops just beneath this ligno-suberized area, giving rise to rapidly dividing cells similar to those of a cambium (figs. 4, 7, 8, 10). The thin walls of these new cells are soon digested, forming a line of cleavage that cuts off the old dry leaf (fig. 4a) and allows it to fall from the twig. When this abscission of the marcescent leaf occurs, the line of cleavage is formed on the proximal instead of on the distal side of the groove.

The abscission layer formed in the petioles of marcescent leaves in early spring is similar to the one formed in the petiole of a leaf that falls in autumn, the most striking difference being the more marked regularity of cell division. The cells appear like a true cambium. The new cells formed become ligno-suberized to form a protective layer over the scar left by the fall of the old dead leaf. It is apparent, therefore, that in the case of marcescence, the leaf is retained simply because an abscission layer fails to form during the autumn.

In some instances development of the abscission layer in the autumn is arrested before it is completed, and the leaf may remain on the branch for some time after the other leaves have fallen. Leaves

remaining on the trees later than January usually reveal little if any morphological evidence of the formation of an abscission layer (figs. 6, 11).

Marcrescent leaves, even on the same plant, do not all fall at once, but once begun, abscission proceeds rapidly and the fall is much more uniform than is the case with leaves falling in autumn. *Quercus coccinea* is the first to become active, shedding its leaves rapidly and uniformly, since the vascular strands of the petioles are small and more easily severed. Although leaves of *Q. velutina* begin to shed at about the same time, the petioles are somewhat larger, and consequently abscission proceeds more slowly. *Q. marilandica* begins to drop its leaves shortly after, and in a manner similar to the two species just mentioned. *Q. rubra* sheds its leaves more slowly than any of the other species. This undoubtedly is due to the fact that the vascular strands of the petioles are large, and the cells in general have heavier walls. The bundles in this species do not always break in the same plane with the other cells, and so commonly leave prominent leaf traces in the leaf scar. In a great many instances a partially formed abscission layer occurs in the petioles of the larger leaves of *Q. rubra*. *Q. alba* is the last of the oaks observed to begin shedding, but once abscission begins, it proceeds rapidly. cursory observations were made on a few species in other genera, including *Fagus grandifolia*, *Ostrya virginiana*, and *Acer saccharum* var. *nigrum*. The behavior of these is similar to that of the species of *Quercus*. The marcescent leaves of *F. grandifolia* remain on the branches long after the buds begin to swell, and in some instances until the new leaves have expanded. A single specimen of *Acer saccharum* var. *nigrum* was observed to retain its leaves until many of the new ones were almost fully expanded.

The writer wishes to express his gratitude to Drs. P. D. STRAUBAUGH and H. S. WOLFE of the department of botany of West Virginia University for numerous suggestions and criticisms during the progress of this work.

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[Accepted for publication October 4, 1930]

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FURTHER EVIDENCE ON THE NECESSITY OF BORON FOR HEALTH IN CITRUS¹

A. R. C. HAYS AND L. J. KLOTZ

(WITH SIX FIGURES)

A previous paper² described some of the physiological and anatomical changes that take place when budded citrus trees are grown for several years in pure quartz sand and are given a culture solution lacking boron.³ Briefly these changes were: corking and splitting of the leaf veins; curling and abscission of leaves; abundant production and premature death of new growth; multiple buds; in extreme cases a splitting of the bark of twigs and trunk followed by exudation of gum, decay of roots, and an accumulation of excessive amounts of carbohydrates in affected leaves. Histological studies showed a degeneration chiefly of the cambium and phloem regions accompanied by the production of gum.

As these results were obtained from studies on a few large budded trees in sand cultures, it seemed desirable to repeat the experiments with great numbers of cultures of seedlings and cuttings. The senior author³ has called attention to the fact that leafy lemon twig cuttings in water cultures may be supplied, from the enamel of the pans, with amounts of boron adequate for healthy growth. Likewise it was pointed out that new 12-gallon glazed earthenware containers used for sand cultures provide sufficient boron for healthy growth when boron is lacking in the original culture solution. Subsequent experiments have shown that when such containers are used for several years they fail to supply boron in amounts adequate for normal growth, as was evidenced by the appearance of all the symptoms typical of boron starvation. When boron is supplied to such cultures the symptoms of deficiency rapidly disappear. The badly affected

¹ Paper no. 232, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² HAYS, A. R. C., and KLOTZ, L. J., Some anatomical and physiological changes in citrus produced by boron deficiency. *Hilgardia* 5:175-197. 1931.

³ HAYS, A. R. C., Boron as an essential element for healthy growth of citrus. *BOT. GAZ.* 89:410-413. 1930.

leaves show but little improvement in appearance when boron is supplied, but absciss less prematurely.

The 2-quart Mason jars in our glasshouse experiments have been in use for over ten years. During the greater portion of this time the jars have been used for experiments with citrus seedlings or cuttings, and although no boron was added to the culture solution, not until about the tenth or present year have any symptoms of boron deficiency been evident. Now, however, practically every plant in each of 80 or more jars shows symptoms of boron deficiency. Figs. 1 and 2 show the effects on leaves and roots. The amount of boron in the seed or in the leaves and twig of the leafy cuttings is sufficient to support normal growth for only a short time.

Leafy lemon cuttings that have grown several years in Swedish enameled mixing bowls of 6-liter capacity, containing a culture solution from which boron was omitted, are now gradually showing more of the symptoms of boron deficiency. Whenever boron is added to the culture solution in concentrations of 0.1 or 0.2 p.p.m., the new growth shows marked improvement regardless of whether the citrus grown is of seedling or cutting origin. The results of our experiences show that it is essential that the glassware, earthenware, or enamelware containers be in use several years prior to their being used in experiments on boron deficiency; otherwise it might be concluded that boron is not an essential element unless the cultures are allowed to grow for several years.

In addition to ordinary leafy twig cuttings, various grafted combinations⁴ of leafy twig cuttings were grown in aged 2-quart Mason jars in a culture solution from which boron was omitted. In making these combinations the three leaves of the leafy cutting used as the stock were removed prior to rooting the stock in sand, and only the cutting represented by the scion was allowed to produce new leaves.

There were seven sour orange (*Citrus aurantium* Linn.), thirteen Rough lemon (var. of *C. limonia* Osbeck), two Spanish bitter orange (var. of *C. aurantium* Linn.), and seven Lisbon lemon (var. of *C. limonia* Osbeck), 3-leaf cuttings. The cutting combinations of scion on stock consisted of two Valencia orange (*C. sinensis* Osbeck) on

⁴ These cuttings were made by Dr. F. F. HALM of the University of California Citrus Experiment Station.

Valencia orange, four Valencia on Eureka lemon (var. of *C. limonia* Osbeck), two Valencia orange on sour orange, four Eureka lemon

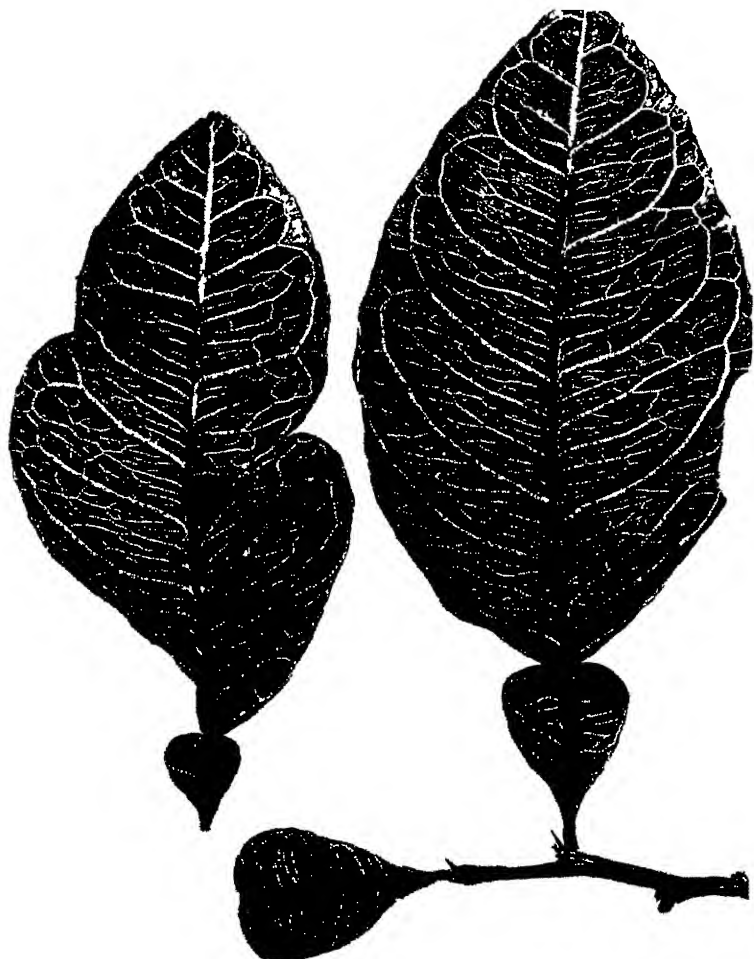


FIG. 1.—Leaves from leafy grapefruit cutting as scion on Eureka lemon cutting as stock: upper surface showing corky split veins of leaves from boron-deficient cultures (bud in axil of attached leaf developed slightly and then died).

on sour orange, three Rough lemon on Eureka lemon, three Marsh grapefruit (*C. maxima* (Burm.) Merrill) on Eureka lemon, one sour orange on sour orange, and two sour orange on Rough lemon. In

each of these, and in many additional cultures designed for other purposes, whenever boron was omitted for several months the leaves showed corky and split veins, multiple buds, cessation of new growth of tops and roots, bulbous enlargement of root tips, and eventual decay of much of the root system. No gum formation has as yet been observed in any of these young cultures. It was evident that insufficient boron was being obtained from the aged Mason jars or as an impurity from the salts used in preparing the culture solutions.



FIG. 2.—Effect of boron-deficiency on roots of sour orange cuttings in water culture: left, affected roots showing stunted bulbous tips and considerable decay; right, normal root system.

The addition of boron as boric acid in an amount sufficient to make a concentration of 0.1 to 0.2 p.p.m. in the culture solution brought about unusually rapid recovery. Fig. 3, showing cultures of lemon seedlings of the same age, demonstrates the necessity for boron and the recovery following the addition of boron. Figs. 4-6 show similar effects with 3-leaf cuttings and with grafted combinations of cuttings.

It has been shown in our previous paper⁵ that where boron was omitted from the culture solution, sugars accumulated in the leaves. This was associated with disintegration of the conducting tissue. Upon the addition of boron to these cultures, there was rapid growth

⁵ See footnote 2.

of new conducting tissue, followed by a decrease in the sugar content of the leaves as recovery progressed.

In the present investigation with sour orange cuttings, determinations of total sugars plus starch in the mature leaves of boron-deficient cultures showed approximately double the amount found in mature leaves of cultures supplied with boron, the values being re-



FIG. 3.—Lemon seedlings showing right, symptoms of typical boron-deficiency, left, recovery showing normal growth following addition of boron.

spectively 11.67 and 5.96 per cent of the dry matter. These results with cuttings in water culture confirm the previous results with budded trees in sand culture. The early resumption and the rapidity of growth, following the addition of boron to cultures previously deficient in this element, may be a consequence of the rapid movement of this large supply of carbohydrates following formation of new conducting tissue.



FIGS. 4-6.—Fig. 4, sour orange cuttings showing. left, curled leaves with corky split veins, typical of boron deficiency; right, new healthy growth following addition of boron (all affected leaves formerly present in culture on right have abscised). Fig. 5, combination of leafy cutting of Rough lemon as scion on Eureka lemon cutting as stock: right, defoliation as result of boron deficiency; left, new growth following addition of boron to culture previously deficient in this element. Fig. 6, combination of leafy cutting of Eureka lemon as scion on sour orange cutting as stock: right, defoliation as result of boron deficiency; left, new growth following addition of boron to culture solution previously deficient in this element.

To throw further light on the causes for the rapid recovery of boron-deficient citrus when given boron, the relative diastatic activity of mature leaves of boron-deficient and boron-supplied cultures was determined. Five hundred-mg. samples of the dry leaf powder and 25 cc. samples of a 1 per cent starch solution were placed in 150 cc. Erlenmeyer flasks and incubated 12 hours at 40° C. The suspension was then filtered and the reducing sugars of the filtrate determined. Using the same procedure, blank determinations of 500-mg. samples of leaf powder in 25 cc. of distilled water were made at the same time. The results obtained were subtracted from those of the first set in order to correct for differences in the intrinsic reducing power of the leaves. The leaves from the boron-deficient cultures showed a slightly greater diastatic activity than those receiving boron in the culture solution, the results being in the ratio of 150 to 138.5 in the case of the sour orange cuttings, and 131 to 120 in the case of the cutting combination of Eureka lemon scion on Rough lemon stock. These results suggest a further reason for the rapid recovery of formerly boron-deficient citrus, and the simultaneous rapid decrease in the carbohydrate content of the leaves.

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[Accepted for publication October 13, 1930]

A NEW METHOD FOR DETERMINING THE PRO-
PORTION OF THE LENGTH OF A TRACHEID
THAT IS IN CONTACT WITH RAYS¹

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(WITH ONE FIGURE)

In the progress of some research work, it became desirable to know just what proportion of the length of a tracheid along the radial face of a piece of softwood is in contact with rays. Obtaining such information through direct measurement of tangential microtome sections, or of the corresponding photomicrographs, is an extremely tedious operation, since a fair average value then necessitates a great number of measurements. This reason made preferable a simplified indirect method that gives an average value directly, and, more important, substitutes the simple operation of counting the structural elements for the laborious and uncertain one of measuring them.

The determinations were made on transverse- and tangential-section photomicrographs that had been enlarged fifty times. For the tangential sections, the number of double walls of tracheids extending across the photomicrograph along the line of intersection of any transverse plane, usually over an actual distance of 0.30 cm., were counted as well as the number of rays cut by this line; for this purpose the "double walls" included the pairs of single walls separated by rays as well as those actually in contact. The number of rays so intersected, divided by the total number of double tracheid walls, gives the average ratio of length of tracheid and rays in contact to the total length of both tracheid-ray contact and tracheid-tracheid contact along the radial faces of the tracheids considered, providing the number counted is not too small. This relationship may be stated in somewhat different terms as follows: The probability of any transverse plane that intersects a tracheid touching a ray cell

¹ Contribution from the Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, maintained at Madison in cooperation with the University of Wisconsin.

will vary directly with the ratio of the combined heights of all rays that are in contact with the tracheid to the total tracheid length.

As a check upon the validity of this relationship, a few determinations were made with the direct-measurement and the count method on the same photomicrographs. In order to increase the accuracy for the direct-measurement method, tangential-section photomicrographs with an enlargement of 300 times were used. The sum of the heights of all the rays in the direction of the tracheid lengths and the sum of all the tracheid wall lengths making up the photomicrograph section were determined. Fig. 1 shows a tangential photo-

TABLE I

RATIO OF LENGTH OF TRACHEID-RAY CONTACT TO TOTAL TRACHEID-WALL CONTACT BY BOTH THE COUNT AND THE DIRECT-MEASUREMENT METHODS, FOR *PICEA SITCHENSIS*

SPECIMEN NO	COUNT METHOD			DIRECT MEASUREMENT METHOD		
	Average number of		Ratio of rays to tracheid walls	Total height of rays (cm)	Total length of tracheid walls (cm)	Ratio of ray to tracheid length
	Rays crossed	Tracheid walls crossed				
1	3 22	16 0	0 201	74 5	367	0 203
	3 48	17 0	0 205	81 9	403	0 203
	2 78	16 0	0 174	66 4	380	0 175
2	3 22	19 9	0 162	77 8	476	0 163
	2 82	17 8	0 158	65 5	426	0 154
	3 17	17 6	0 180	69 9	400	0 175

micrograph of *Picea sitchensis*² with the heights of the rays indicated, and the first line of table I shows the results obtained from this section. Because of the relatively few tracheids and the smaller number of rays intersected by a single transverse line, counts of the rays cut by a number of transverse lines were made at intervals of 1 cm. on the original photomicrographs (fig. 1), and the average was taken so as to give more accurate results. Table I gives the results for three different photomicrographs of each of two different specimens. The ratios of length of tracheid-ray contact to total tracheid-wall contact obtained by the two methods agree very well.

Although fig. 1 and table I show that the results obtained by the

² Names of species in this article follow SUDWORTH, G. B., Check list of the forest trees of the United States, their names and ranges. U S Dept. Agric. Misc. Circ. 92.

two methods are comparable, they do not show the real saving in labor of the count method. When the counts are made upon enlargements of lower power, which was done for the data given in table II, the results demonstrate that the count made across a single intersecting line gives a fair average value (for 75 to 100 tracheids), and that a single line is therefore sufficient. It is obvious that such a count can be made much more readily than the measurement in the

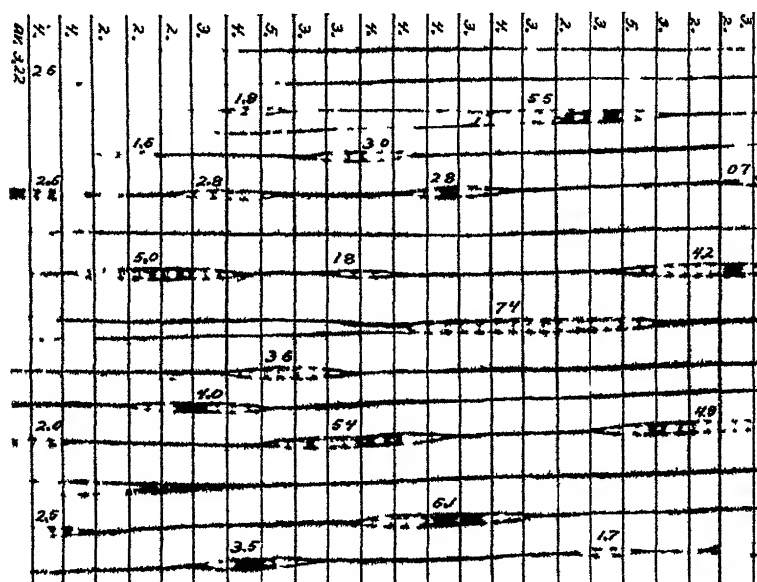


FIG. 1.—Cross-section of softwood, ruled to show statistical principle underlying the new method, which in practice needs only a count made along a single straight edge, $\times 150$

direct method of the total height of the rays touching a single tracheid.³ In general the count data should also give a better average value, since there is more chance of the properties of a single tracheid varying from the average than the properties of the great number of tracheids along the line of intersection.

Data can also be obtained by the count method from transverse sections. In them the rays are continuous, and can be counted easily.

³ A recent article (CLARKE, S. H. *Forestry* 4:42 1930) describes a recording micrometer used in conjunction with a projection apparatus that would greatly simplify the direct-measurement method, but still the indirect method should be less laborious.

TABLE II

PROPORTION OF LENGTH OF TRACHEID IN CONTACT WITH RAYS DETERMINED BY
COUNT METHOD FOR VARIOUS SOFTWOODS ON PHOTOMICROGRAPHS
OF 50 TIMES ENLARGEMENT

NAME OF SPECIES	TRANSVERSE SECTION				TANGENTIAL SECTION				AVG- AGE OF BOTH GROUPS OF RATIOS
	Dis- tance exam- ined (cm)	No tra- cheids	No rays	Ratio	Dis- tance exam- ined (cm)	No. tra- cheids	No rays	Ratio	
<i>Abies amabilis</i>	{ 3	72	15	208	.3	72	15	208	204
	{ 3	72	14	194	3	75	16	213	
	{ 3	75 ¹	15	200	3	73	15	205	
	{ 3	70	14	200	3	70	14	200	
<i>Abies balsamea</i>	{ 3	110	19	173	3	113	19	168	168
	{ 3	111	18	162	3	114	20	175	
	{ 3	114	19	167	.3	109	18	165	
<i>Abies concolor</i>	{ 3	86	14	163	3	89	15	169	177
	{ 3	88	15	170	3	88	17	193	
	{ 3	89*	16	179	3	84	16	190	
<i>Abies magnifica</i>	{ 3	74	20	270	3	72	20	278	278
	{ 3	75	21	280	.3	70	19	271	
	{ 3	79	22	279	3	73	21	288	
<i>Abies nobilis</i>	{ 3	75	12	160	3	94	14	149	154
	{ 3	78	12	154	3	99	15	152	
	{ 3	81	13	160	3	101	15	149	
<i>Chamaecyparis lawsoniana</i>	{ 3	109	9	083	3	112	11	098	089
	{ 3	106	10	094	3	112	10	089	
	{ 3	110	9	082	3	107	10	093	
<i>Chamaecyparis thyoides</i> ..	{ 3	106	12	113	3	101	12	119	122
	{ 3	105	12	114	3	105	14	133	
	{ .3	106	14	132	3	105	13	124	
<i>Juniperus virginiana</i>	{ .28	124	16	129	3	131	16	122	118
	{ .28	127	15	118	3	129	15	116	
	{ .28	125	14	112	3	126	14	111	
<i>Larix laricina</i>	{ 3	104*	25	240	3	103	26	252	250
	{ 3	100	24	240	.3	94	26	277	
	{ 3	97	23	237	3	98	25	255	
<i>Larix larix</i>	{ .3	83	19	229	.3	95	21	221	229
	{ .3	90*	21	233	.3	91	22	242	
	{ .3	86	20	233	3	96	21	219	
<i>Larix occidentalis</i>	{ 3	83	15	181	.22	53	10	189	187
	{ 3	87*	16	184	.22	50	10	200	
	{ .3	84	16	190	.22	53	10	189	

* Summerwood.

TABLE II—Continued

NAME OF SPECIES	TRANSVERSE SECTION				TANGENTIAL SECTION				AVERAGE OF BOTH GROUPS OF RATIOS
	Distance examined (cm)	No tra-cheids	No rays	Ratio	Distance examined (cm)	No tra-cheids	No rays	Ratio	
Libocedrus decurrens	3	121	16	132	3	113	16	142	.141
	3	122	17	139	3	116	18	155	
	3	125	17	136	3	114	16	140	
Picea glauca.	3	91	20	220	3	95	21	221	.221
	3	95*	23	242	3	100	22	220	
	3	91	18	198	3	107	24	224	
Picea mariana	3	101	16	158	3	97	16	165	.167
	3	102	18	176	3	103	17	195	
	3	110*	19	173	3	102	17	167	
Picea sitchensis	3	100	20	200	3	98	17	173	.185
	3	108	18	167	3	99	18	182	
	3	100	18	180	3	92	19	207	
Pinus banksiana	3	116	24	207	3	109	22	202	.205
	3	121*	25	207	3	104	23	221	
	3	116	23	198	3	109	21	193	
Pinus contorta.	3	106	14	132	3	107	14	131	.139
	3	112*	16	143	3	102	14	137	
	3	105	15	143	3	103	15	146	
Pinus echinata (wide growth rings).....	3	80	13	163	3	83	12	145	.152
	3	83*	14	169	3	84	11	131	
	3	88	14	159	3	83	12	145	
Pinus echinata (narrow growth rings)...	3	80	13	163	3	78	13	167	.152
	3	88*	14	159	3	79	12	152	
	3	83	11	133	3	78	11	141	
Pinus lambertiana	3	64	5	078	3	65	6	092	.086
	3	69*	5	072	3	68	6	088	
	3	65	5	077	3	65	7	108	
Pinus monticola...	3	99	8	081	3	92	8	087	.089
	3	92*	9	098	3	94	8	085	
	3	93	8	086	3	95	9	095	
Pinus ponderosa.....	3	101	15	140	3	94	13	138	.144
	3	109	17	156	3	103	14	136	
	3	109	16	147	3	104	14	135	
Pinus resinosa...	3	104	16	154	3	104	13	125	.141
	3	100	14	140	3	96	12	125	
	3	99*	16	162	3	92	13	141	

* Summerwood.

TABLE II—Continued

NAME OF SPECIES	TRANSVERSE SECTION				TANGENTIAL SECTION				AVERAGE OF BOTH GROUPS OF RATIOS
	Distance examined (cm)	No tracheids	No rays	Ratio	Distance examined (cm)	No tracheids	No rays	Ratio	
<i>Pinus taeda</i>	3	95	18	189	3	101	16	158	170
	3	92*	18	196	3	96	14	146	
	3	94	17	181	3	105	16	152	
<i>Sequoia sempervirens</i> (small cells)	3	117	19	162	3	116	17	147	145
	3	126+	19	151	3	116	16	138	
	3	127	17	134	3	118	16	136	
<i>Sequoia sempervirens</i> (large cells)	3	62	10	161	3	59	10	160	165
	3	60*	10	167	3	61	10	164	
	3	60	10	167	3	56	9	161	
<i>Sequoia washingtoniana</i>	3	99	20	202	3	98	18	184	185
	3	97	18	186	3	102	18	176	
	3	100*	17	170	3	103	20	194	
<i>Thuja occidentalis</i>	3	125	11	088	3	113	11	097	095
	3	120	11	092	3	116	11	095	
	3	124	13	105	3	120	11	092	
<i>Thuja plicata</i> (wide growth rings)	3	130	14	108	3	105	14	133	120
	3	129+	16	124	3	117	14	120	
	3	129	13	101	3	113	15	133	
<i>Thuja plicata</i> (narrow growth rings)	3	106	11	104	3	111	15	135	126
	3	108*	13	120	3	100	14	140	
	3	103	13	126	3	97	13	134	
<i>Tsuga canadensis</i> ...	3	105*	21	200	3	131	20	153	178
	3	94	18	191	3	129	21	163	
	3	97	19	196	3	128	21	164	
<i>Tsuga heterophylla</i> ...	3	94	14	149	3	90	14	156	158
	3	97+	16	165	3	96	16	167	
	3	97	15	155	3	88	14	159	

Summerwood.

Dividing the number of rays that any tangential line intersects by the number of double tracheid walls along that line again gives the ratio of tracheid-ray contact length to that of total tracheid-wall. A comparison of results obtained on transverse- and tangential-section photomicrographs is given in table II, three separate counts being made on different parts of each photomicrograph. The transverse-section data give further a comparison between the values of the ratio for summerwood and for springwood.

Although the results given in table II are insufficient to permit drawing general conclusions regarding the ratio of tracheid-ray contact length to total tracheid-wall contact length, because only one or two specimens from each species have been included, they point to several interesting relationships. The ratio is practically the same for both summerwood and springwood, indicating that there is little relationship between the local density of the wood and this ratio. The results show also that values for the ratio obtained from different parts of the same small specimen are substantially constant. This is not surprising, since each value is an average for a great number of tracheids and thus should at least approximate the over-all average. The agreement between the results for the transverse and the tangential sections is good, and is all the more indicative because the photomicrographs examined were picked from the files at random, and the sections may not have come from the same block. In three instances results are given for two different sets of specimens of the same species. The agreement for *Pinus echinata* is far better than can generally be expected, while for *Sequoia sempervirens* and *Thuja plicata* the agreement is good, indicating that the variation within a single species may not be great. Further, the variation between the values for different species of the same genus in general is less than the over-all variation.

Summary

1. A simple method has been suggested for determining the ratio between the length of tracheid-ray to total tracheid-wall contact that requires only the counting of structural elements.
2. A comparison between the results obtained with this method and with the direct-measurement method is given.
3. The values for this ratio obtained for all the different species of softwood examined range from 0.072 to 0.288. Values of the ratio for a given species vary considerably less, and, for different parts of the same specimen, still less. There is no significant difference between the values for summerwood and for springwood.

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[Accepted for publication October 27, 1930]

CURRENT LITERATURE

BOOK REVIEWS

Taxonomy of flowering plants

This journal has already noted texts in plant taxonomy by DIELS and by HITCHCOCK (BOT. GAZ. 81:342. 1926), and also by SWINGLE (*ibid.* 86:115. 1928). The appearance of another work in this field is now recorded, the noteworthy text by JOHNSON.¹ This work is monumental in many respects, although it abounds in chronological, orthographic, lexicographic, and other types of inaccuracies to an extent greater than can be justified, even in a first edition. The author displays a broad and balanced perspective, and appears to have kept foremost in mind the needs of students and the demands of taxonomic pedagogy. His style is dignified and conservative, yet as interesting and attractive as the limitations of the subject matter permit. An outstanding feature of the volume is the surprising wealth of original drawings. These number several thousand, grouped into nearly five hundred aggregations modestly called figures, but which might with greater propriety have been treated as full-page plates. These illustrations were drawn by the author himself, and combine to an exceptional degree morphological accuracy with artistic excellence. They abound in glimpses of the more intimate or even cryptic details of floral structure. It is safe to predict that for many years to come these alone will be sufficient to insure the use of JOHNSON's book by teachers and students, not only in plant taxonomy but also in allied branches of botany.

Part I consists of 150 pages, and is devoted to flower analysis and fundamental principles. In chapter I, the science of taxonomy is treated briefly in historical retrospect. Subsequent chapters deal with such subjects as nomenclature, genus and species, phylogeny, homology and analogy, the flower, fruit, seed, seedling, inflorescence, and vegetative characters. Part II opens with chapter X and includes the remainder of the book. It is devoted to systematics. Chapter X contains many valuable generalizations and discusses the approach to classification. It is followed in chapter XI with various practical suggestions on species and specific distinctions, genera, generic characters and distinctions, fruit and seed characters, leaf characters, comparisons, construction of keys, field studies, and collecting for a herbarium. The rest of the text proper, which is by far the larger part of the entire book, is given over to the *Dicotyledones* and *Monocotyledones*, group by group. Here the author has drawn freely upon WETSTEIN, ENGLER and PRANTL, BENTHAM and HOOKER, and other writers for technical material pertaining to the different groups. The volume concludes

¹ JOHNSON, A. M., *Taxonomy of the flowering plants*. pp. xxi+864. figs. 478. Century Co., New York. 1931.

with a glossary and a bibliography. The glossary is somewhat full for a work of this size, but a cursory inspection shows various points meriting criticism. Thus, *amplexicaule* is the spelling given to a supposedly English adjective, while Gray's Manual, the Century Dictionary, etc., prefer *amplexicaul*; *decussate* is defined as "alternating in two rows, in zigzag manner, on opposite sides of the stem," which is hardly to be reconciled with "alternating in pairs at right angles" as in Gray's Manual, the Century Dictionary, and other authoritative works. The bibliography is planned in a helpful way, since it is subdivided into sections dealing with floras, manuals, and special treatises; manuals and handbooks of trees and shrubs; works on grasses, including cereals; works of a popular nature; works on teratological phenomena; works on ecology, phytogeography, natural history; works on forests and forestry; works on geology and paleontology; histories of botany; botanical periodicals in English; ecological journals; additional works (textbooks) on ecology; and recent textbooks on systematic botany. The usefulness of the bibliography is impaired, however, by many inclusions of insignificant works and omissions of important ones, not to mention mistakes in dates, proper names, etc. Surely a second edition can be made to represent taxonomy with much greater fidelity if it shall first have been given a vigorous and detailed re-editing. Such re-editing would prove particularly valuable if to the task could be brought a taxonomist of much practical experience in herbarium and library research, as distinguished from that in class-room and field work.—E. E. SHERIFF.

Tall-grass prairie in Nebraska

An unusually thorough and valuable description of some of the climatic and soil conditions of the Nebraskan tall-grass prairie is made available by WEAVER and HIMMEL.² Observations and results of instrumentation are presented, derived from a study that extended from 1915 to 1928, for an upland station and for one in the lowland, both in the vicinity of the city of Lincoln. A brief characterization of the two samples of climax vegetation is followed by descriptions of the two soils, including the usual data from mechanical and chemical analyses. Then the following fluctuating environmental conditions are described, with tables and graphs: amount of precipitation and its seasonal distribution; soil-moisture content at several depths (expressed as gravimetric percentages above the hygroscopic limit); air temperature; soil temperature (at several depths); air humidity (expressed as relative humidity); wind movement (treated only superficially); and evaporation (nearly complete weekly records of water loss from standardized white cylindrical porous porcelain atmometers for the summers of 1916, 1917, 1919, 1920, 1921, 1922, 1924, 1925 and 1926). Although some of the methods of instrumentation and of computation and integration might conceivably be improved, it is remarkably fortunate that routine

² WEAVER, J. E., and HIMMEL, W. J., The environment of the prairie. Bull. no. 5. Conservation and Survey Division, Univ. of Nebraska. 1931.

procedure was almost wholly maintained throughout these many seasons. Consequently the extensive data form a nearly homogeneous series and give a presumably fair picture of the conditions for eastern Nebraska in the present climatic period. Differences between the different summers are discussed. Physiologists and ecologists, and students of applied botany also, may note that our present lack of any well authenticated and feasible procedure for evaluating solar radiation as a climatic feature is tacitly emphasized, for that important climatic condition is not considered at all.

The paper is primarily descriptive and the discussions are not at all exhaustive. An important general conclusion, in which most students of ecological climatology will probably concur readily enough, is that the amplitudes of the natural fluctuation of the non-water conditions of the region in question, from week to week, and from summer to summer, are not sufficiently great to be markedly influential on this type of vegetation, but that the natural fluctuation of the moisture conditions of air and soil do have amplitudes wide enough to bring these conditions almost into the category of limiting factors. Slight differences in moisture conditions appear to account for the notable vegetational differences between upland and lowland prairie vegetation in general, and between the performance of the component plants in different years. If the climate were to become only slightly drier or wetter the vegetation would probably show prompt and conspicuous adjustment, but it would probably require much more pronounced change in temperature conditions to bring about notable alteration in the vegetational aspect. This is in accord with indications that may be derived from a vegetation map of the United States.—B. E. LIVINGSTON.

Handbook of plant nutrition and fertilizer science

A work of unusual magnitude and outstanding value in the field of plant nutrition has been prepared under the able leadership of HONCAMP,³ the director of the Rostock Experiment Station. The work consists of two large volumes, one on plant nutrition, the other on fertilizer science. It is difficult to do justice to a great contribution such as this in a limited review.

Realizing the specialized development of the field covered, HONCAMP secured the collaboration of an extensive group of experts, fifty of whom were enlisted in the effort. Each important section has been prepared by one whose experience and interests best fitted him for the task. The work is well illustrated, with 90 figures in the first volume and 285 in the second.

Volume I contains ten chapters. It opens with an interesting history of plant nutrition, from the time of ARISTOTLE down to HELLRIEGEL'S famous experiments. The second chapter deals with the constituents and composition of the plant body, and the third with the cycle of the main nutrient elements in

³ HONCAMP, F., *Handbuch der Pflanzenernährung und Düngerlehre*. Vol. I, *Pflanzenernährung*. 8vo. pp. xv+945. Vol. II, *Düngemittel und Düngung*. 8vo. pp. xii+319. Julius Springer, Berlin. 1931.

nature. Chapter IV is devoted to the physiology of metabolism in plants. The next chapter considers the soil as the habitat and nutrient reservoir for plant growth. Chapter VI deals with the yield laws, chapter VII with methods of investigation, water and sand cultures, etc., and chapter VIII with methods of field investigations. The last two chapters discuss the evaluation of fertilizer experiments and the determination of nutrient deficiencies of soils. Here one finds illuminating discussions of MITSCHERLICH's methods and the seedling method of NEUBAUER and SCHNEIDER. As this is discussed by NEUBAUER himself, it is of course an authoritative account.

Volume II presents the fertilizer problems in nine chapters, the first of which is again a general review of the field, and the second an account of naturally occurring fertilizer materials. Chapters III and IV consider the manufactured fertilizers and their application. Chapter V discusses the specific fertilizer requirement of certain crop plants, root crops, grains, legumes, oil and fiber plants, meadows and pastures, vegetables, vineyards and orchards, hops, tobacco, etc. Chapter VI is devoted to forest fertilizer problems, chapter VII to those of moor and heath, and chapter VIII to those of ponds. The last chapter considers the relation of fertilizers to plant disease and insect problems, and to the control of weeds. There is an enormous amount of valuable information gathered into these volumes, and the work will prove very helpful as a reference and guide to the present status of plant nutrition studies. Those who are fortunate enough to be able to use them will appreciate the immense amount of time and labor which have been required of the editor, collaborators, and publishers in making such a vast source of information available to the investigator.—C. A. SHULL.

Soil microorganisms

An interesting book has been written by WAKSMAN and STARKEY⁴ describing the microbes of the soil, and giving their relation to soil processes and plant growth. Botanists know all too little about the microscopic organisms of the soil. As brought out by the authors, the soil is not just an inert mixture of disintegrated rock and organic matter, but is teeming with a microscopic population of plants and animals. The activities of these microorganisms are absolutely essential for the growth of higher plants, and indirectly for the development of all higher life, both plant and animal. By way of illustration, to be available for plant growth, nitrogen must be present in the soil in the form of nitrates or ammonium salts; but nitrogen in these forms is never present in the soil in amounts of more than a few pounds per acre. Microorganisms of the soil are constantly changing the organic nitrogen to ammonium salts and nitrates, thus keeping in circulation some of the essential elements.

The book is divided into ten chapters. Chapter I is concerned mainly with

⁴ WAKSMAN, S. T., and STARKEY, R. L., *The soil and the microbe: an introduction to the study of the microscopic population of the soil and its rôle in soil processes and plant growth*. 8vo. pp. xi+260. figs. 87. John Wiley & Sons, New York. 1931.

the processes of soil formation and plant nutrients. Chapters II and III discuss the microbes of the soil and their distribution and activity. Chapter IV considers the work of the microorganisms in decomposing the organic matter of the soil. Chapters V, VI, and VII are concerned with the rôle of microorganisms in the transformation of the soil nitrogen and of the mineral substances. In chapter VIII various interrelationships between the plant and the microorganisms of the soil are considered, including nitrogen fixation, nodule formation by legumes, mycorrhiza, etc. Chapter IX deals with the effects of various factors, such as organic matter, cultivation, liming, and reaction, in modifying the soil population. Chapter X is of the nature of a summary, giving the importance of microbes in soil fertility.

The book is not intended as an exhaustive treatise of the subject, but as a compact description of the microorganisms of the soil and their activity. It serves this purpose admirably. It is written in a clear, convincing style. One characteristic contributing to its clarity and readability is that when a point is in controversy, the theory backed by the most evidence is given, without much reference to other possible theories. While the research worker would probably desire a fuller discussion of other possible theories, it must be admitted that the plan adopted has its value. A list of the most important books is given at the end of each chapter. No citations are referred to by number in the text of the book. The authors have given an excellent description of the microorganisms of the soil and their relationships to soil fertility.—S. V. EATON.

Plant ecology

Four years have elapsed since the publication of McDOUGALL's textbook in plant ecology, and now the second edition has appeared.⁵ The order of presentation is precisely the same as that of the first edition, and there has been little change in the textual material. About one-half of the chapters are identical with those of the previous edition, and in the other chapters the changes were made by inserting a brief paragraph or two to state some additional facts. The most extensive modification of this kind occurs in chapter XII, where the author has added two paragraphs dealing with atmospheric humidity. There are five new illustrations, and a number of new references have been placed in the lists at the end of each chapter. In his preface to the new edition the author states that "the material has been carefully revised in order to bring it up to date," and it is therefore somewhat disappointing to find no hint of some of the more recent work; for example, no reference has been made to MAXIMOW's⁶ interpretation of xerophytism.—P. D. STRAUSBAUGH.

⁵ McDOUGALL, W. B., *Plant ecology* (2d ed). pp. xii+338. Lea & Febiger, Philadelphia. 1931.

⁶ MAXIMOW, N. A., *The physiological nature of drought resistance of plants*. Proc. Internat. Congress Pl. Sci. Ithaca, New York. 1926. 2:1169-1175. 1929.

THE BOTANICAL GAZETTE

October 1931

FACTORS AFFECTING GROWTH FROM THE FOLIAR MERISTEMS OF *BRYOPHYLLUM CALYCINUM*

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(WITH FOUR FIGURES)

Introduction

At the base of each marginal notch of the leaf of *Bryophyllum calycinum* is a group of cells which, under favorable conditions, may produce an entire new plant. A study correlating the activity of these cells with the functions of those adjacent, and with the several plant organs, demonstrated, according to LOEB, certain mass relations. These relations he stated were the only precise logical explanation for regeneration in plants and animals.

Studies by REED (31), OSSENBECK (26), KAKESITA (19), and others have presented evidence contrary to the findings of LOEB. It seemed advisable to attempt a reinterpretation of the available data, to test the validity of current hypotheses, and to study certain of the chemical and enzymatic changes which accompany growth from the foliar meristems.

Materials

The plant used, *Bryophyllum calycinum* Salisb. (*Br. germinans* Blanco; *Br. pinnatum* Kurz.), is the same species as that employed by GOEBEL, DE VRIES, REED, OSSENBECK, and others. Five collections from the following sources were used; (1) the conservatory at Golden Gate Park, California; (2) a private garden in Santa Barbara, California; (3) the University of California, Berkeley; (4) a

collection at the University of Wisconsin; and (5) materials of the same lot as those used by REED (31), obtained from Professor H. H. BARTLETT of the University of Michigan. Collection 5, bearing the label "University of Michigan Botanic Gardens 1761," was selected as standard for these studies.

Varietal differences

There are indications of varietal differences of a degree sufficiently great to account for the lack of uniformity in observations made by investigators in the past. Plants from collections 4 and 5, when placed in a darkroom ventilated with greenhouse air, under conditions which were abnormal only in the absence of light, showed in 10 days a random distribution of proliferation from notches of all leaves except those of the upper three nodes. No proliferation was observed on any plant of collection 1 or 3 under identical conditions. These observations may explain why OSSENBECK (26) was unable to verify positive results reported by REED regarding the activating effect of darkness. Root systems of all plants used in the present study were carefully examined, and all were found to be healthy and functioning.

Another constant varietal or strain difference was manifest in the rooting potentialities of petioles from plants in lots 1 and 4 on the one hand, and of those in lot 5 on the other hand. At no time during many trials, which involved the use of a wide variety of media, were any roots produced from the petioles of plants composing lots 1 and 4. In every experiment with leaves from plants of lot 5, roots were obtained from some of the petioles. It was apparent that leaves from different clones of the same species differ in the distribution of toti-potential cells.

Morphology

The single recent publication regarding the anatomy of the leaf notches is a short and rather inconclusive statement by BEALS (3). Camera lucida drawings of sections of notches are presented, with the conclusion that the roots and shoots "arise from the division of small phloem cells near the vegetative points or notches of the leaves." Morphological studies have shown the leaf to be bifacial and to possess no palisade. Stomata are present on both surfaces and are surrounded by subsidiary cells.

There is a probable chemical difference in cells which respectively compose the abaxial and adaxial halves of a leaf. Young leaves stained with Flemming's triple stain after fixation with formalin-acetic-alcohol appear to be divided through the center by a region of vascular tissue. On each side are small parenchymatous cells which although they do not noticeably differ morphologically from one another, behave differently toward stains. Such differences in staining have also been noticed on preparations made with simple stains, such as gentian violet.

A definite polarity of dorsiventral nature is observable in the proliferating notch. Roots invariably arise from the abaxial side of the leaf regardless of its position, and leafy shoots always arise from its adaxial surface.

Studies were made of the anatomy of the developing leaf to determine whether or not the meristems which give rise to new roots and shoots are formed normally in the development of the leaf, or only following unusual stimulation of the plant. No effort was made to trace the condition further than to situations which exist in leaves one-eighth of an inch in length. At this stage of development the leaf will not proliferate, even under the most favorable experimental conditions. In leaves this size marginal, subepidermal meristems composed of a number of nearly isodiametric cells are noticeable. No observation has been made in the present study which would suggest that cells which compose the foliar meristems are at any time more than embryonic. Thus it does not appear that proliferation in *Bryophyllum* involves the changing of differentiated cells to the embryonic condition before other changes occur. It does appear, however, that the notch meristems are differentiated extremely early in the normal development of the leaf, much as axillary buds are formed in the normal development of the stem. During the time in which the surrounding cells differentiate into the tissues of the mature leaf, the cells which are later to form the new plants remain in an embryonic condition, although their numbers increase, as may readily be observed. Well developed vascular regions are found adjacent to the meristems. Such a coincidence may have suggested to BEALS that the meristems have their origin in these vascular regions, but no evidence has yet been presented to demonstrate beyond doubt

that these veins and meristems may not have wholly separate origins.

Current hypotheses of regeneration

Proliferation from leaf notches of *Bryophyllum* has been explained by hypotheses of four types, involving: (1) formative stuffs; (2) inhibitive substances; (3) altered nutrient relations; and (4) anaerobic respiration.

According to the hypothesis of formative stuffs, applied to this problem by LOEB, specific hormones are synthesized in the leaves of *Bryophyllum* plants, irrespective of attachment of the leaf to the plant. When the leaf is a functional part of the plant, these hormones are removed by the growing points of the stem and the roots; hence an accumulation of sufficient quantities of hormones to activate the notch meristems cannot occur. If the leaf is separated from the plant, however, the hormones accumulate and stimulate the production of new plants.

To establish the hypothesis of formative stuffs, it must be shown that proliferation occurs only in the absence of root and shoot growing points, and that it never occurs in their presence. Experimental data are at hand to demonstrate the inadequacy of such a hypothesis. Numerous cases of proliferation which have occurred in the presence of functioning root and shoot apices will be cited later. The present study has also verified the experimental results of REED (31), that a leaf may be removed from the influence of both root and shoot apices for a time without stimulating the marginal notches to activity. Moreover, LOEB's theory fails to explain the following:

1. Attached leaves of unmutilated plants have been observed to proliferate by REED (31), RENICH (32), SMITH (35), BRAUN (7), FYSON (12), and even by LOEB himself (24). Such cases of so-called regeneration have been observed by the writer in material used at California and Wisconsin, and in the field, under natural conditions, in Honolulu (fig. 1).

2. Numerous experiments^{*} have demonstrated that the shoot

^{*} All experiments were conducted in a greenhouse in which there were no artificial gas outlets, and in which plants extremely sensitive to ethylene or coal gas showed not the slightest injury.

apex which, according to LOEB, removes the formative stuffs from the leaves and thus keeps them dormant, may be removed without stimulating proliferation. Growth from the axillary buds (axils of leaf as a whole) replace the apex.



FIG. 1.—Proliferation of attached leaves of young vigorously growing plants, growth occurs from notches in contact with moist soil, no special treatment used (photography by courtesy of J. P. BENNETT).

3. Root growth has been inhibited for long periods by encasing the roots in plaster of Paris casts. Shoot growth was limited simultaneously without causing the growth of foliar meristems.

4. While leaves were attached to plants, growth was induced at the notches by the following means: (1) immersion of leaves in water

(WAKKER 38 and REED); (2) making a single incision through the midvein of the leaf (GOEBEL 13); (3) darkness (REED 31)*; (4) crown-gall inoculations (SMITH 35)*; (5) building up of moist sand to the lower surfaces of attached leaves (REED)*; (6) warm bath (KAKESITA 19); (7) hydrogen atmosphere for 48 hours (KAKESITA)*; (8) chemical injections (KAKESITA); (9) etherization (GOEBEL 13)*, (10) prolonged saturation of soil (Experiment 1)*; (11) extended drought (Experiment II)*; (12) ringing plants by removal of fleshy cortex and phloem (GOEBEL 13)*; (13) wrapping stems tightly with thread (Experiment III)*; (14) injections of tissue juice (Experiment IV)*.

EXPERIMENT I.—An observation of FYSON (12) that a number of plants growing in open fields in India showed proliferation from the leaf notches after a long rainy period suggested that the same results might be brought about experimentally by excessive moisture about the roots. Accordingly vigorous potted plants were placed in glass vessels of boiled water to a depth sufficient to cover the soil by 2 inches. Heavily paraffined wrapping paper was fastened securely over the top of the vessel and around the base of the stem, which protruded centrally from the dish. Within one week roots were growing from the notches of the leaves of all plants used. Later roots were also produced on the stems above the water level.

EXPERIMENT II.—Drought under natural conditions has been noted by many to have an activating effect upon buds in general. This fact, together with evidence that ether exerted a stimulating effect upon the leaf meristems of *Bryophyllum*, seemed to indicate that insufficient water might have a noticeable effect on the activity of the notches. Several plants were therefore set aside to dry for 5 weeks. Control plants of the same age and variety were placed in a similar situation but received water daily. Within the experimental period almost every leaf of the dry plants except those of the top two nodes showed root development. Many repetitions of this experiment gave similar results.

EXPERIMENT III.—Around each of six small plants 4 months old 8-10 yards of white silk thread was wound tightly at an internode

* These methods of stimulating proliferation have been used successfully in the present study.

some distance above the soil. During the following 5 months, increase in girth of the stems left a considerable constriction at the point about which the thread was wrapped. Above this constriction the stem was of more than normal diameter. Shoot growth took

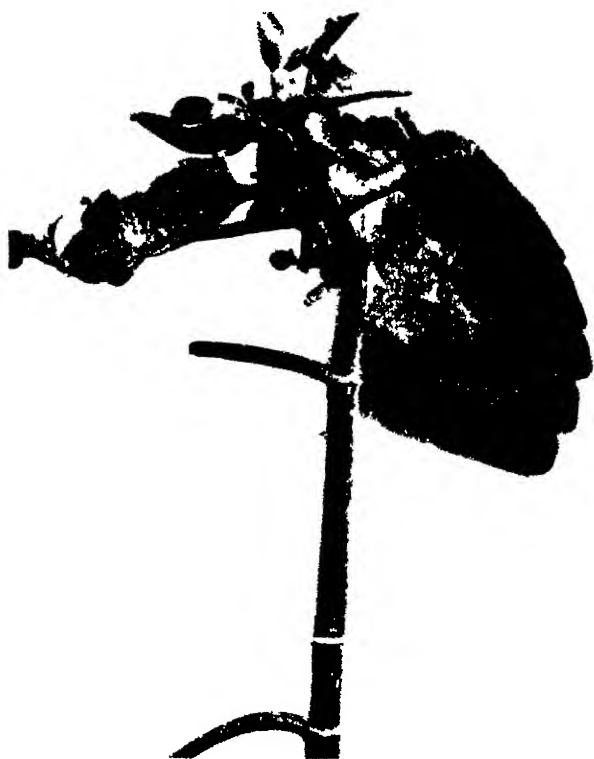


FIG. 2.—Proliferation of apical leaves of vigorously growing plant induced by tightly wrapping stem with thread; some leaves removed at time of photographing.

place from the leaves above the constriction; on leaves of control plants no such growth occurred (fig. 2).

EXPERIMENT IV.—While there is an extensive literature regarding the effects of accumulations of hormones upon the activity of foliar meristems of *Bryophyllum*, it is pertinent to note that an experimental study to demonstrate the actual presence of such hormones has never been made. For such a study, tissue adjacent to

and including the notches was removed from a total of 150 notch areas. The leaves for these notch areas were separated from the plants for a period long enough to show slight proliferation. Sister leaves (leaves opposite the ones removed) were marked with tags, and a similar number of dormant notches with adjacent tissue were removed. The dormant and active notches were separately frozen. Each was then placed in a mortar with a small amount of quartz sand, and ground to a pulp. Ten cc of water was used to wash the pulp of each into hard glass beakers. Hydrogen-ion determinations of each extract were made, and the beakers then set in a refrigerated room for 72 hours at a temperature of 10° C. At the end of this time

TABLE I
EFFECT ON DORMANT LEAF MERISTEMS OF INJECTION OF TISSUE JUICE
EXPRESSED FROM ACTIVE AND DORMANT NOTCHES

NOTCH TYPE USED AS INOCULUM	pH OF INOCULUM	DATE OF INOCULATION	DATE OF OBSERVATION	RESULTS
Active	6.15	2/6/30	3/10/30	} Small shoot growth from number of notches
Dormant	6.10	2/6/30	3/10/30	

the water and residue were stirred together and the mixture centrifuged. Small amounts of the supernatant liquid were injected into tissue adjacent to dormant notches of leaves of vigorous plants. The injection was made by means of a hypodermic syringe, which was of glass except the needle. Table I shows the results of the experiment.

There is no evidence at hand to demonstrate that the water-soluble fraction of dormant and active notches differs in any respect with regard to the presence of activating hormones. The incompleteness of this line of experimental evidence is thoroughly recognized, however, but it may be said that the LOEB hypothesis of formative stuffs is entirely without verification in any experiment of the present study. A theory of inhibiting substances, such as LOEB later advocated (21), is also untenable for similar reasons. In all events the present theory of hormonal control of proliferation is unsatisfying, since to be of intrinsic value it must deal with material changes of demonstrable types.

On the basis of mass relationship studies, LOEB (24) stated that photosynthetic materials of the usual nature instead of hormones are probably the cause of proliferation:

To complete the proof that we are dealing here with the action of the quantity of material produced by assimilation it remains to be shown that the results described occur only in the presence of light, while in the dark the production of roots and shoots in an isolated leaf of *Bryophyllum* is negligible. . . . This explains in part why in the normal plant no regeneration of roots and shoots occurs in the leaf. In the normal plant all material is used either for growth of the leaf or growth of the stem apex, roots, and stem itself, as will be seen later

The results of the writer's experiments do not lend themselves to this interpretation. First, proliferation from foliar notches not only does occur in the dark, but a higher percentage occurs in the dark during a given period than occurs in constant illumination (sunlight supplemented by two 500-watt Mazda lamps 3 feet above, constantly burning), and, second, leaves or stem pieces which have been kept in constant darkness for 47 days will produce roots and shoots without subsequent exposure to light. Thus in experimental plants the absence of photosynthetic activity for 7 weeks, during which time two internodes elongated in each plant, there still remained sufficient materials of proper type to permit proliferation from the foliar notches.

KAKESITA (19) indicated that intermediate products of anaerobic respiration were the cause of proliferation of leaf meristems. Briefly stated, he demonstrated that notches may be activated by the Molisch warm-bath treatment, which treatment, according to BORESCH, induces intramolecular respiration. An atmosphere of hydrogen for 48-72 hours was found sufficient to cause activation by an accumulation of chemical materials of a type formed during alcoholic fermentation by yeasts. These results appeared to be verified by chemical injections of intermediate and end products of alcoholic fermentation.

A critical review of KAKESITA's data and methods has been made. First, owing perhaps to the preliminary nature of the paper, KAKESITA's methods were not given in detail. The method of applying the Molisch warm-bath treatment may have been one of two ways. The entire plant may have been submerged in such a manner

that the soil was water-logged, or it may have been inverted over the water so that the entire plant above the soil line was treated while the soil still contained no more than the original amount of water. Such a variation of method may be of crucial importance in the results obtained. Presumably, in the absence of a statement to the contrary, the soil was saturated. The method of "carefully considering a supply of fresh air" for the plants subsequent to treatment is not elucidated, yet this factor is of prime importance for a correct interpretation of the effects due to the period of treatment. The same criticism may be made of the studies to determine the effect of an atmosphere of hydrogen on proliferation. These plants were "covered with bell jars and were given a full supply of fresh air." The conditions subsequent to treatment are too vaguely presented.

If anaerobic respiration does cause proliferation, a relatively inactive gas, such as nitrogen, should produce a positive effect when used as an atmosphere to exclude oxygen. To avoid confusion in distinguishing results which might be due to the absence of oxygen from those which are due to an accumulation of carbon-dioxide, a flow of gas sufficient to keep the concentration of carbon-dioxide at a minimum must be used. In my work special apparatus was designed to make possible such flow of gases, and to eliminate difficulties due to insufficient moisture. Fig. 3 is a diagram of the ventilated moist chamber unit which was used in the present series of experiments.

In November, 1929, an attempt was made to study the effect upon the leaf notches of plants of atmospheres of air (1) minus carbon-dioxide; (2) minus carbon-dioxide and oxygen (nitrogen was used); and (3) unaltered except that it was washed by bubbling through distilled water in constant light, constant darkness, and intermittent illumination (12 hours per day of each). The apparatus consisted of nine moist chambers of the type diagrammed in fig. 3. These were arranged in series of three chambers each, and were separated from one another by gas washing bottles of 500 cc. capacity. Series *A* consisted of three chambers in which the plants were treated for 124 hours with air washed through a 60 per cent solution of potassium hydroxide to remove the carbon-dioxide. Series *B* consisted

of three similar chambers in which the plants were treated for the same period with air washed through bottles of distilled water.

Plants of series *C* were treated with nitrogen washed through alkaline pyrogallate solution (pyrogalllic acid 22 per cent, KOH 60 per cent). In each series the three conditions of illumination were maintained as illustrated in fig. 4. Constant darkness was obtained by painting the bell jars with three coats of dull black Japalac. Constant light was obtained by using a 500-watt Mazda lamp suspended 2 feet above the center control chamber. Intermittent light was provided by means of fiber board hoods, shown in fig. 4. These were painted black on the inside and white on the outside, and were of such a height that they permitted a flow of gas through the inlet tubulatures of the moist chambers without interference. As shown in fig. 4,

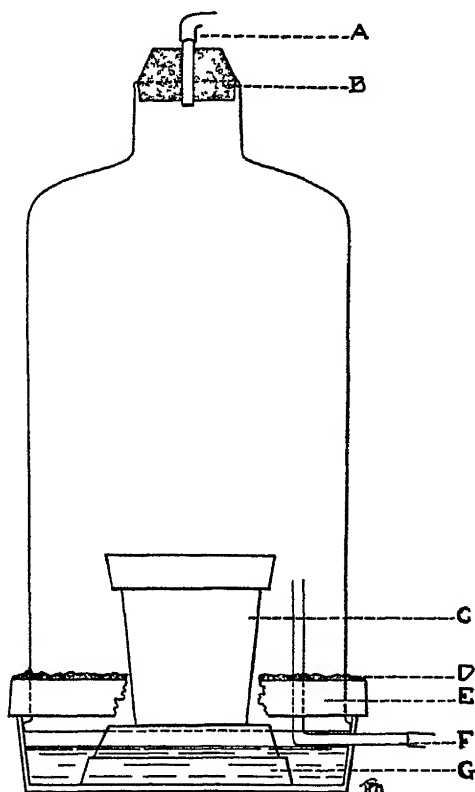


FIG. 3.—Diagram of ventilated moist chamber unit: *A*, glass tube inlet with rubber connection to gas source; *B*, rubber stopper securely shellacked in place; *E*, large clay saucer saturated with paraffin and sealed tightly to adjoining bell jar by seal *D* (composed of plasticine painted over with paraffin); *C*, potted plant resting on inverted clay saucer *E* to prevent saturation of soil by water in *E*; *F*, outlet tube connected either to wash bottle or to water-jet vacuum pump.

the bases were buried in builder's sand to a depth of 2 inches. Beneath the hoods the outlet tubulatures passed through the sand in such manner that they were not disturbed by removal

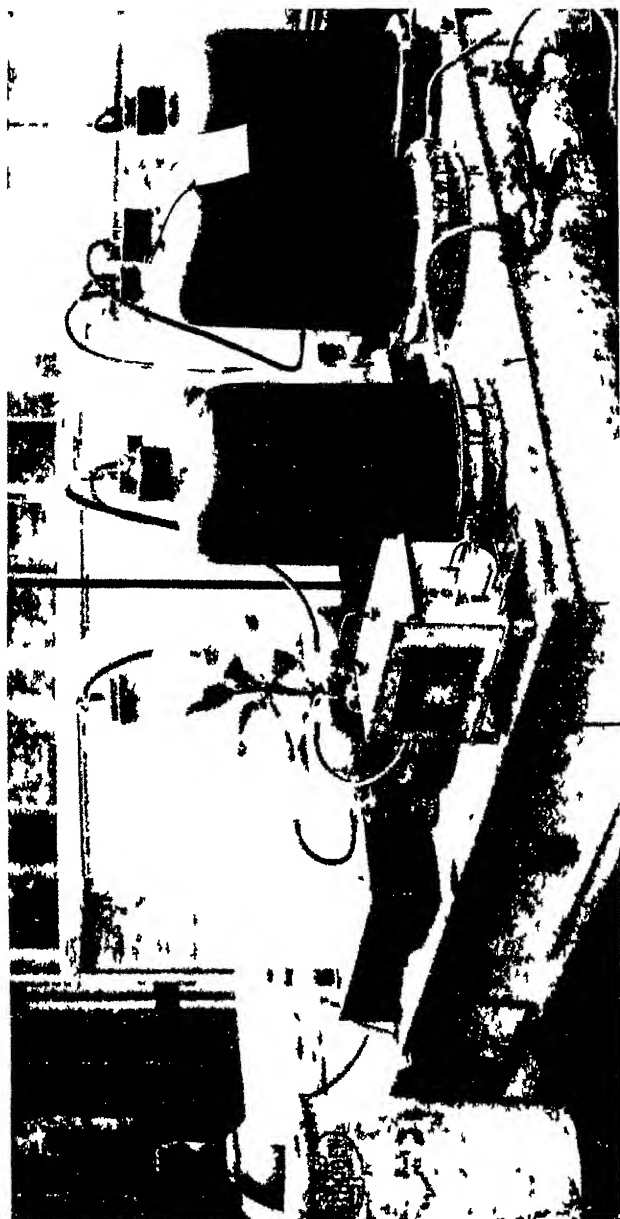


FIG. 4.—Illustration of apparatus used

of the hoods. Temperatures during the 124-hour gas treatment were recorded automatically, and varied from 67° to 83° F. After the 124-hour period the nitrogen was discontinued, the pyrogallate was replaced with clean wash bottles of distilled water, and the series was attached to a water jet vacuum pump (fig. 4). In a similar manner the KOH solution of series A was displaced with distilled water. During the remaining days of the experiment the temperatures reached the abnormally high point of 90° F. on the eleventh day, and a low point of 54° F. on the seventeenth day. The average temperature was $\pm 70^\circ$ F. During the entire experimental period gas was moving through the chambers at the rate of 6–7 liters an hour, thus a complete change of atmosphere presumably occurred every 2 hours.

In contrast with the gas experiments by KAKESITA, the following differences should be borne in mind: (1) nitrogen instead of hydrogen was used, (2) the period of treatment in artificial atmospheres was extended to 124 hours in contrast to the 72-hour period of KAKESITA, (3) a method was used to eliminate high concentrations of carbon-dioxide by constantly changing the atmospheres once every 2 hours, (4) three conditions of light were observed, (5) nine separate combinations of gas and light factors were made possible simultaneously, (6) the methods made possible a separate determination of the effect of absence of oxygen and absence of carbon-dioxide on proliferation, (7) the limiting effect of absence of moisture was removed.

A repetition of this experiment, in which the period of exposure to nitrogen was reduced to 72 hours, gave results similar to those of the longer exposure. In both instances the condition which should favor intramolecular respiration, namely, an atmosphere of nitrogen, did not noticeably increase the occurrence of proliferation from the leaf notches of the experimental plants. In all conditions of light and in all atmospheres, some proliferation occurred. Moisture seemed to exert a controlling influence.

The Molisch warm-bath treatment has been reported by KAKESITA to be a successful means of stimulating growth from foliar notches of *Bryophyllum*. OSSENBECK previously reported negative results from similar attempts. In the present study, unmutated vigorous plants suspended over a water bath for 10 hours at a tem-

perature varying from 30° to 35° C., in such a way that the soil was not wet, showed no activating effect of the warm bath. It seems that the Molisch bath in itself is not the cause of proliferation in *Bryophyllum*, but that conditions following the treatment may cause the activation, especially very moist conditions.

Several means of causing proliferation do not appear to support the KAKESITA hypothesis: (1) activation of leaf notches by cooling roots under conditions that cause no permanent injury to the roots (OSSENBECK 26); (2) darkness (REED 31)*; (3) cutting the midvein of a leaf in such a way that under similar conditions wounding of large areas of leaf tissue between the major veins exerted no influence (GOEBEL)*; (4) drought. All these environmental conditions may have other effects in common. They would conceivably have an appreciable effect upon carbohydrate synthesis, and it seems that many of the observed effects of changed environmental conditions may be more readily explained on the basis of their effect upon photosynthesis than as causing anaerobic respiration.

Since experiments performed by the writer and by others fail to support two of the fundamental bases of KAKESITA's hypothesis, it is felt that this hypothesis cannot be strongly supported as a general explanation of proliferation of foliar meristems of *Bryophyllum*.

Enzyme studies

Of the standard methods available for activating dormant buds, tubers, and seeds, the following have given positive results in stimulating dormant notches of *Bryophyllum*: treatment with ether, mechanical stimulation by needle pricks, ringing of the stem with a knife, tightly binding the stem with thread, and drought. Other methods which have been ineffective in the present study are the Molisch warm-bath treatment, injection of ethyl alcohol, organic and inorganic acids, and injection of the POPOFF (30) "cell stimulants." The consensus of opinion seems to be that the activation of dormant structures by these means is due to their effect on the enzymes, either directly or indirectly (2, 6, 9, 11, 17, 18, 27, 37).

A complete series of experiments to throw light on the enzyme relations of activated and dormant notches and tissue adjacent to

* These experiments were successfully repeated in the present study.

these in the leaves of *Bryophyllum* were planned. At the present time only a preliminary statement can be made regarding the carbohydrases and catalase.

LOEW (25) described the enzyme catalase. Two types of catalase were recognized, alpha and beta. Alpha catalase is probably a compound of the soluble catalase beta with a nucleo-protein, while beta is an albuminose and can be liberated from the combination by the action of very dilute alkali. Studies to determine the relative activities of catalase from plant tissues must be planned with great care to overcome many sources of error (1, 15, 16, 29).

The apparatus used was a modification of the APPLEMAN apparatus (1). This modification was as follows: (1) the use of glass stop-cocks throughout; (2) coloring the water used in the manometer by the addition of a trace of fast green in alcohol; (3) the insertion of a U-tube of soda lime between the reaction chamber and the manometer to remove any carbon-dioxide; and (4) the use of a mechanical shaker. This shaker was operated off the agitator in the constant temperature bath in which the reaction chamber, a 500 cc. round bottom, Pyrex flask, was immersed.

The hydrogen peroxide used was Merck superoxol. This was diluted with conductivity water to give a concentration of about 3 per cent. The actual concentration was determined by the use of 0.1 nitrogen potassium permanganate standardized against sodium oxalate by the method described in TREADWELL and HALL. The peroxide used was brought to a pH of 6.8 by the addition of sodium hydroxide. Brom thymol blue was used as an indicator.

The method of preparing the tissue was standardized as follows: leaves were ground through a food chopper, inserted in washed muslin bags of double thickness, and placed in a hydraulic press. A pressure of 600 units on the pressure gauge was maintained for 1 minute. The juices were collected and to each sample was added 4 cc. of redistilled water for each gram of original leaves used. The mixture was permitted to stand in a constant temperature bath at 20° C. for 1 hour. After stirring the material to insure homogeneity, various amounts were then pipetted out for the determinations.

Table II gives results characteristic of the activity of catalase in leaves with dormant or active notches. Each set of figures repre-

sents an average of two readings, which checked within 5 per cent. Some of the leaves used were dormant, and others active (separated from the plant for 1 week).

The writer's data on catalase activity are parallel with those of BONNS (6) on etherization effects on *Gladiolus* bulbs. Catalase activity in germinating seeds has been reported to be greater than in

TABLE II
RELATIVE ACTIVITY IN JUICE EXPRESSED FROM LEAVES OF BRYOPHYLLUM

NOTCH CONDITION	pH	TISSUE SAMPLE (cc.)	H ₂ O ₂ SAMPLE (cc.)	OXYGEN FORMED (cc.)	REACTION TIME (MIN.)	PERCENTAGE DRY WEIGHT
Dormant .	5.9	0.5	5	3.8	10	10.42
Active..	6.18	0.5	5	3.6	10	11.85
Dormant .	5.9	1.0	5	9.33	10	
Active....	6.18	1.0	5	10.0	10	
Dormant..	5.9	2.0	5	20.4	10	
Active....	6.18	2.0	5	20.2	10	
Dormant .	5.9	4.0	5	34.1	10	
Active...	6.18	4.0	5	32.5	10	
Dormant	5.9	7.0	5	32.8	5	
Active....	6.18	7.0	5	32.8	5	
Dormant .	5.9	10.0	5	39.4	5	
Active... .	6.18	10.0	5	34.2	5	

Dry weight determinations were made of tissue samples after chopping in food grinder just before placing in press.

A blank of 5 cc. H₂O₂ for 10 minutes gave a negative reading of 0.5 cc.

similar dormant seeds. Rest-breaking agents are said to stimulate activity in potato tubers. Proliferation in *Bryophyllum* shows no similar relationship.

CARBOHYDRASES

The standard method adopted for these studies was to use, in an Erlenmeyer flask of suitable size, 35 cc. of a 2 per cent solution of carbohydrate with 10 cc. redistilled water and 5 cc. of tissue juice. To each flask toluol was added as a preservative, and the mixtures were placed for 20 hours in a constant temperature darkroom at 24° C. After this period the reducing sugars in each flask were determined.

METHOD OF PREPARING SUBSTRATES.—*Maltose*: 5 gm. maltose hydrate (Pfanstiehl) dried in vacuum oven for 1 hour, plus redistilled water to bring the total volume up to 250 cc., gave the standard 2 per cent solution.

Sucrose: 5 gm. Difco sucrose plus redistilled water to a total volume of 250 cc., gave a 2 per cent solution.

Dextrin: dried in an oven 1 day at 40° C., 5 gm. dextrin (Pfanstiehl) precipitated by alcohol, plus redistilled water to a total volume of 250 cc., gave a 2 per cent solution.

Soluble starch: 5 gm. of soluble starch placed in a flask with 175 cc. redistilled water and boiled for 15 minutes with a reflux condenser; cooled in cold water and washed quantitatively into a 250 cc. volumetric flask. Brought up to volume this procedure gave a 2 per cent solution.

METHOD OF PREPARING TISSUE.—Several leaves were removed from vigorous plants, cut into fourths, and placed in clean glass germinators lined with clean moist filter paper. The sister leaves were each labeled and left attached. After 3 days the leaves showed proliferation from the notches. The excess moisture was removed from the surface by means of blotting paper, and the leaves weighed and ground in a food chopper. This and similar material prepared from the dormant sister leaves was frozen and the juices extracted in a hydraulic press.

METHOD OF DETERMINING REDUCING SUGARS.—The method used is a variation of the SHAFFER and HARTMANN (34) method; the principal change is the dilution of all solutions used to one-fourth the concentration recommended by them.

Table III gives a typical series of results from studies to determine the relative activity of sucrase, maltase, dextrinase, and amylase in leaves with dormant notches or active ones. In this table the value of the reducing sugars in the flasks of each substrate which contained tissue juice from leaves with dormant notches was arbitrarily selected as representing 100 per cent, for the sake of an easy comparison of the value.

When each lot of experimental materials (substrate plus juice) was ready for determination, the material was neutralized with KOH. Each flask of material was then cleared with neutral lead

acetate and de-leaded with potassium oxalate. The supernatant liquid was made up to volume, and 20 cc. aliquots were added to, and heated with, Fehling's solutions. Thus, since comparable flasks were treated simultaneously, inactivation of the enzymes of the comparable flasks was accomplished in the normal procedure of determining the reducing sugars present. Negative results reported by BONNS (6) on the activation of carbohydrases in etherized tissues may have been the result of autoclaving the reaction chambers to stop the

TABLE III
RELATIVE ACTIVITY OF VARIOUS CARBOHYDRASES SHOWN BY QUANTITY
OF REDUCING SUGARS PRESENT AFTER ACTION OF
ENZYMES FOR 20 HOURS

SUBSTRATE	TISSUE TYPE	pH	REDUCING SUGARS IN MG. PER FLASK	REDUCING SUGARS PERCENTAGE COMPARISONS
Sucrose	{Dormant	5 05	78 8929	100 00
	{Active	5 35	102 0537	129 36
Maltose	{Dormant	5 05	202.8184	100 00
	{Active	5 35	270 8828	135 55
Dextrin	{Dormant	5.05	43.7435	100 00
	{Active. ...	5 35	56 8493	129 96
Soluble starch	{Dormant	5 05	46.0209	100.00
	{Active	5 35	46.8373	101 77

enzyme activity at the end of the reaction period. Masking of the enzyme action by inversion of the substrates at the time of autoclaving is suspected.

From the data in table III it is clear that, under conditions maintained during the experiment, an increase in activity of maltase, sucrase, and dextrinase occurred, but no considerable increase in the activity of amylase was found. It is possible that this enzyme was also activated, but the extended reaction period may have masked differences which would have been apparent at an earlier time.

Chemical relations

The enzyme changes which occur during proliferation should effect a change in the chemical composition of the leaves in which

such proliferation is occurring. Determinations of total carbohydrate, total nitrogen, total phosphorus, and carbohydrate fractions are to be reported. It should be stated, however, that much additional work is required to determine many of the chemical changes which occur concomitantly with activation of foliar meristems of *Bryophyllum*.

METHODS

PREPARATION OF TISSUE.—Leaf tissue to be analyzed was first dried at 80° C. Leaves which had been separated from the plant long enough to show small roots from the notches constituted one lot. Sister leaves which had not been separated from the plant constituted the other lot. Each lot was pulverized in a fine burr mill. The powder thus obtained was placed in glass stoppered weighing bottles, where it was held for future use.

Phosphorus determinations were made according to BRIGG's (8) modification of the BELL and DOISEY (4) method. Comparisons of color were made by the aid of a Klett colorimeter. Total nitrogen determinations were made by the usual Kjeldahl method modified to include nitrate nitrogen. Total carbohydrates were determined by hydrolyzing powdered leaf tissue for 2 hours in 2.5 per cent HCl in a reflux condenser. Following hydrolysis the material was neutralized, cleared, dealeded, and reducing sugars determined as described. The separation of carbohydrate fractions was as follows.

A. REDUCING SUGARS.—Two gm. of powdered leaf tissue was boiled with 150 cc. of 70 per cent ethyl alcohol for 15 minutes. The mixture was filtered and the filtrate evaporated to dryness and then taken up in water. The resulting solution was cleared and dealeded. The reducing sugars were determined by the modified SHAFFER and HARTMANN method.

B. GLUCOSIDES.—An aliquot of the aqueous solution prepared in A was treated with 2.5 per cent HCl for 5 hours on a boiling water bath. Following neutralization, the solution was brought to volume and the reducing sugars determined.

C. TOTAL SUGARS.—An aliquot part of the aqueous solution prepared in A was hydrolyzed with 2.5 per cent HCl for 15 minutes on a boiling water bath. Following neutralization, the reducing sugars present were determined.

D. DEXTRINS, SOLUBLE STARCH, STARCH.—The residue from filtration in A was strongly boiled with redistilled water for 15 minutes. It was then hydrolyzed with pangestin for 2 hours, during which time it was occasionally stirred. The material was rapidly filtered and the residue saved for E. The filtrate was hydrolyzed with 2.5 per cent HCl for 2.5 hours; then neutralized, cleared, dealeded, and the reducing sugars present determined.

TABLE IV
CHEMICAL COMPOSITION OF COMPARABLE LOTS OF PROLIFERATING
AND NON-PROLIFERATING LEAVES OF BRYOPHYLLUM

DETERMINATIONS	PROLIFERATED 20 LEAVES (GM)	NON-PROLIFERATED 20 LLAVES (GM)
Original weight	104 1	100 4
Weight when proliferated	60 45	
Dry weight .	12 00	11 20
Dry weight percentage ..	8 67	8 96
Total nitrogen..	1 24 per 100 gm dry weight	1 33 per 100 gm dry weight
Total carbohydrates ..	54 88 per 100 gm. dry weight	60 82 per 100 gm. dry weight
Total phosphorus .	154 94 mg. per 100 gm. dry weight	129 0 mg per 100 gm. dry weight

E. GUMS, SOLUBLE PECTINS, HEMICELLULOSES.—The residue from filtration in D was hydrolyzed in 2.5 per cent HCl for 5 hours in a reflux condenser; subsequently the material was centrifuged, neutralized, cleared, and dealeded. The reducing sugars were determined.

Twenty leaves from the central and lower regions of plants 1 year old were activated by separation from the plant and by being placed on a stretcher of dry cheese cloth. Sister leaves were tagged on the plants as controls. As soon as growth appeared from the notches of the separated leaves, the two groups were assembled, weighed, and prepared for chemical study. In table IV it will be noted that the proliferating leaves lost approximately 40 per cent of their weight during the period of activation. The data presented in this table show in comparison that in proliferated leaves there are decreases of approximately 10 per cent in carbohydrates, and approximately 6 per cent in nitrogen, and a relative increase of 17 per cent in

phosphorus. It is apparent that no considerable change in carbohydrate/nitrogen ratio necessarily accompanies activation.

To determine whether or not chemical changes in leaves, such as those just noted, may be regarded as the cause of activation of foliar meristems, a study of the chemical constitution of non-proliferating leaves was made. All leaves selected were of an age to permit proliferation. Table V is a summary of the constitution of upper and

TABLE V
CHEMICAL COMPOSITION OF YOUNG AND OLDER NON-
PROLIFERATING LEAVES OF BRYOPHYLLUM

DETERMINATIONS	CONSTANT LIGHT		NATURAL LIGHT	
	Upper leaves	Lower leaves	Upper leaves	Lower leaves
Wet weight grams	81 9	77 3	105 1	112 72
Dry weight grams	8 55	12.40	13 20	16 30
Percentage dry weight...	10 41	16 04	12 65	14 46
Phosphorus per 100 gm. dry weight....	178 93 mg.	80 00 mg.	176 48 mg.	104 17 mg.
Total nitrogen per 100 gm. dry weight.....	1 6215 gm.	0 7745 gm.	1 5205 gm.	0 8835 gm.
Total carbohydrates per 100 gm. dry weight... ..	21 48 gm.	42 33 gm.	32 83 gm.	42 11 gm.

lower leaves from healthy plants, one lot of which was exposed to constant illumination, whereas the comparable lot received sunlight only.

Between the younger and older leaves of a plant which displays no proliferation, a greater difference in chemical composition is found than between two lots of sister leaves, one of which has active notches and the other dormant ones. However, the differences between proliferating and non-proliferating leaves from the same plant are greater than either the differences between two lots of non-proliferating young leaves, or the differences between two lots of non-proliferating older leaves from separate plants.

In order to force small plants for experimental use, 200 plants 6 weeks old were placed under constant illumination (sunlight supplemented by the light of five 300-watt Mazda bulbs suspended 3 feet above the pots. After an exposure of 12 weeks, a majority of the plants showed small shoots growing from a number of notches.

In not a single case was a root to be found associated with these shoots. Proliferation caused by mutilation usually evidences root growth prior to the formation of shoots.

From a study of the composition of those leaves in constant illumination showing proliferation, contrasted with leaves from plants

TABLE VI

COMPOSITION OF LEAVES OF BRYOPHYLLUM WITH ACTIVE AND DORMANT NOTCHES; CARBOHYDRATE FRACTIONS EXPRESSED AS GM. REDUCING SUGARS PRESENT, FOLLOWING HYDROLYSIS, PER 100 GM. DRY WEIGHT

PRODUCTS	TISSUE TYPE	CARBOHYDRATE PRESENT
Reducing sugars	{ Active Dormant	3 76 2 76
Glucosides.	{ Active Dormant	3.98 2 86
Total sugars	{ Active Dormant	4 00 2 64
Dextrins, soluble starch, starch	{ Active Dormant	17 64 26 70
Gums, soluble pectins, and hemicelluloses	{ Active Dormant	3 02 10 26
Total carbohydrates	{ Active Dormant	32 36 45 22
Total nitrogen per 100 gm. dry weight	{ Active Dormant	1.85 1.71

of the same variety and age which had not been exposed to constant illumination, the following relationships were demonstrated: in leaves with active notches there is (1) an increase in reducing sugars, glucosides, and total sugars; (2) a considerable decrease in dextrins, soluble starch, and starch with a reduction of nearly one-third in gums, soluble pectins, and hemicelluloses (table VI).

Discussion

In a complex field of many variables, such as the one presented by the living plant, the concept of limiting factors of the BLACKMAN

(5) type may more completely take into account the kaleidoscopic situations than the concept of a single dominating factor. Indeed, a more favorable approach to a valid explanation of proliferation in many cases seems to be an analysis of factors which limit activation under natural conditions. Especially does this appear to be advisable when it is recalled that such contrasting factors as constant light or constant darkness, drought, or excessively high humidity produce the same end result.

Chemical constitution may be a limiting factor, for under suitable environmental conditions, leaves removed from the upper nodes show activation of notches more than 3 weeks later than leaves removed from the lower nodes. Moreover the oldest leaves of large plants often turn yellow and are finally excised. Repeated attempts to bring about proliferation of these yellowed leaves have been unsuccessful. Immaturity in this case cannot constitute a limiting condition, for younger leaves of the same plant will proliferate.

Changes of osmotic concentration seem important in proliferation, for many of the means of activating the foliar meristems appear to increase the osmotic concentrations in cells adjacent to the meristems, and doubtless of them. Such changes are brought about by water-logging the soil, drought, ringing the stem, cutting the leaf midvein, activation by ether, and stimulation by the action of *Phytomonas tumefaciens*. Furthermore, studies of carbohydrate changes in proliferating leaves display a higher concentration of osmotically active materials in these than in non-proliferating leaves.

Attention is called to the increased activity of hydrolytic enzymes, an accompaniment of proliferation. Substances which have been shown to stimulate enzyme activity also induce foliar proliferation. It is considered probable that, whatever the ultimate cause of proliferation may be, it is the result of new conditions which arise owing to environmental changes or injury.

Conditions which favor activation of foliar meristems may be of a type unfavorable to continued growth of the new organs. An example of such a situation is the activation of notches by drought or ether. Short exposures stimulate activity, but longer ones have a lethal effect. Once new growth is started, it must be supported by

suitable food from one source or another. It is here that LOEB's mass laws seem to apply with certain limitations. He states:

Equal masses of sister leaves of *Bryophyllum calycinum* produce in equal time, under similar environmental conditions, approximately equal masses of roots and shoots regardless of the number of roots or shoots formed (except that a more moderate number of shoots formed may possibly permit a more complete utilization of the material furnished by the leaf than if only one shoot is formed).

It is worth while to examine LOEB's data, to determine the extent to which the mass relation may become a universal statement for the particular phase of proliferation to which it applies. DOSTAL (10) performed a number of experiments with *Scrophularia nodosa*, *Bryophyllum crenatum*, and *Circaea intermedia* which demonstrated that even in *B. crenatum* the relationship does not hold; indeed, departures of approximately 50 per cent from the mass law were reported to occur. Furthermore, the data contain figures in contradiction to LOEB's statement (23) that "It was found that the mass of shoots regenerated in two sets of halved stems was in exact proportion to the mass of leaves attached to the stems."

In the materials used by DOSTAL, LOEB's mass relationships do not hold; rather it seems that the smaller the amount of leaf present, the greater the mass of regeneration per gram dry weight of attached leaves.

The possibility of stimulation of notch meristems by many means has been demonstrated. After the production of new plants has been demonstrated by whatever means, the rate of growth of the new structures will be in proportion to the availability of necessary foods, other conditions being similar. The availability of foods would seem to depend on more than the ultimate mass of foods to be drawn upon. The condition of the foods, the relative availability of the proper enzymes, the facility of translocation, and many other factors which are not taken into account by LOEB's theory, will undoubtedly enter as factors in determining the rate of growth of the newly developing plantlets.

Summary

1. Varietal differences which have been found in *Bryophyllum* may account for many of the contradictions in earlier studies.

2. The meristematic cells of the notches arise at a very early age; in fact, while the leaf is still largely embryonic.

3. A dorsiventral polarity in the proliferating leaf was observed, roots invariably arising abaxially and shoots adaxially in the leaf.

4. The abaxial and adaxial halves of young leaves display a difference in staining reaction to Flemming's triple stain and to certain simple stains. This difference may be correlated with polarity of proliferation.

5. Experimental data are presented which introduce difficulties inexplainable by LOEB's hypotheses of formative stuffs and inhibitive substances.

6. Experiments performed by the writer and by other investigators fail to support two of the fundamental bases of KAKESITA's hypothesis of regeneration.

7. Leaves kept in the dark for a period of 47 days will produce roots and shoots from the marginal notches without subsequent exposure to light. Thus it appears that insufficiency of photosynthate in the leaves before being placed in the dark was not an inhibiting factor as suggested by LOEB.

8. An accumulation of oxidized substances incident to wounding is apparently not an activating agent in proliferation.

9. An apparent correlation exists between conditions which cause a change in carbohydrate synthesis, or in the movement of such photosynthate and proliferation.

10. By quantitative chemical means maltase, sucrase, and dextrinase showed activation amounting to an increase of almost one-third in proliferating leaves over controls of sister leaves.

11. Catalase activity was not particularly increased by conditions which cause activation of foliar meristems.

12. Total phosphorus determined by BRIGGS' modification of the BELL and DOISEY method showed a relative increase of 17 per cent in leaves with active notches compared with sister leaves with resting notches.

13. Quantitative chemical analysis showed that under different conditions total nitrogen may be greater or less in leaves with active notches than in control leaves with resting meristems.

14. Quantitative determinations showed the following relationships existing between the young and older leaves of plants all notches of which are inactive: (1) a difference of 45 per cent in phosphorus content from upper leaves to lower leaves; (2) a difference of 50 per cent in total nitrogen, decreasing from upper to lower leaves; (3) a difference of 97 per cent in total carbohydrates, increasing from younger to older leaves tested.

15. A quantitative comparison of carbohydrates in proliferating leaves with those of similar non-proliferating leaves showed the following differences: in leaves with active meristems there is (1) an increase in reducing sugars, in glucosides, and in total sugars; (2) a considerable decrease in dextrans, and a reduction of nearly one-third in gums, soluble pectins, and hemicelluloses.

16. A reinterpretation and restatement of LOEB's mass laws have been made on the basis of his data and of data presented by DOSTAL.

The writer gratefully expresses appreciation to Dr. B. M. DUGGAR for the many criticisms and helpful suggestions received in the execution of the research here reported, and to Dr. R. M. HOLMAN for interest in the problem and for materials.

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[Accepted for publication February 4, 1931]

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ECOLOGICAL SURVEY OF THE BELLA COOLA REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 419

BLANCHE McAVOY

(WITH FIVE FIGURES)

Introduction

The Bella Coola Valley is an east and west valley lying wholly in the coast mountains of British Columbia, at the latitude of $52^{\circ} 21'$. It is the landward extension of a series of channels, Fitzhugh, Burke, and North Bentnick, which cut through the Coast Range from the Pacific Ocean north of Vancouver Island. These channels are typical fiords whose grandeur rivals that of the fiords of Norway. They range from one to three miles wide, with nearly parallel sides and with few indentations or constrictions. Their almost perpendicular walls rise from the water's edge precipitously, and in places culminate in snow capped peaks. These mountain sides drop off just as abruptly beneath the surface of the water as they rise sheer above it, and in some places 300 fathoms have been measured and deeper places may occur.

The study recorded in this paper was made during the summers of 1926 and 1928.

GEOLOGY

Concerning the geology of the Coast Mountains, LINDGREN (6) states:

At the end of the Jurassic or the beginning of the lower Cretaceous intrusions began on a large scale along the coast accompanied by marked uplift: magmas of great volume were intruded forming a great chain of batholiths now exposed by erosion all along the Pacific from lower California to Alaska. In comparison with this intrusion all earlier and later phenomena fade into insignificance. The magmas crystallized into coarse granitic rock but its average composition is not that of granite but of a granodiorite. The rocks are uniformly gray or whitish.

SPENCER (9) believes that the ranges are remnants of an uplifted and much dissected peneplain that was once continuous with the

belt of interior plateaus. He assigns their elevation to post-Eocene times. DOLMAGE (2) says that the Coast Mountain system consists of batholiths that are 100 miles in width. He states also (3) that in the Bella Coola region Table Mountain and Canoe Crossing Mountain, which are about 30 miles east up the Bella Coola Valley, mark the boundary between the Coast Mountains and the interior plateaus.

In regard to the rivers that move westward through the Coast Mountains, SPENCER (9) is of the opinion that they were antecedent to the uplift of the mountains and have maintained their westward course during the rise of the mountains.

During the Pleistocene the valley glaciers moved westward. Glacial grooves on the south side of North Bentnick arm, not far from the mouth of the Bella Coola River, and grooves at the top of Canoe Crossing Mountain show that the ice moved in a westward direction and was at least one mile deep. DALY (1) says that the Scandinavian region was depressed by the load of ice during the Pleistocene, that both North America and Europe sank under the load of ice, and that since that time the continents have been rising slowly. Small rocky benches along the coast of British Columbia may be evidence of a postglacial rise of the depressed coast.

CLIMATE

Places on or near the coast have their greatest rainfall in the winter, as compared with the summer maximum for places in the interior. Also the coast has an abundant rainfall as compared with the meager rainfall of the interior. Near the coast local conditions may have a great effect on the amount of rain. This effect is shown in the well known differences between Vancouver (58.76 inches) and Capilano Creek (129.46 inches). These two places are less than 10 miles apart. Bella Coola with 46.2 inches and Ocean Falls with 144.21 inches, despite the fact that they are only about 50 miles apart in a direct line, also illustrate this fact. At Bella Coola the greatest rainfall occurs in October and November, with 8 inches as the November average. This heavy rain in the valley, together with the melting of early snow in the mountains, causes a yearly autumn flood in the Bella Coola River.

The average monthly temperatures for Bella Coola vary from 26° F. for January to 61° F. for July for a 10-year interval. At the eastern end of the valley the temperatures are lower in winter and higher in summer than they are at the western end. The amount of rainfall decreases so much at the eastern end of the valley that in some cases gardens are irrigated. No official figures are available.

In summer the Bella Coola Valley is well lighted and the days are long, but in winter there are a few weeks when the sun does not rise high enough to shine over the mountains, and at those times the sun can be seen only when it passes notches in the mountains.

Bella Coola Valley

The Bella Coola River is formed by the junction of the Whitewater and the Atnarko rivers, just east of Table Mountain. From here it flows in a westerly direction for about 40 miles, where it empties into North Bentnick arm. The Whitewater comes from glacial fields, and many of the smaller streams that empty into the Bella Coola River from the south carry large amounts of glacial débris. Like most streams that carry too heavy a load, the Bella Coola River is a braided stream at the western part of the valley. For most of the distance the main channel is close to the foot of the mountains that border the north side of the valley. During the summer months the river is white and thick with glacial silt, but in the winter months the water is clear and less in amount.

The mouth of the Bella Coola River is on the south side of the eastern end of North Bentnick arm, and the Necleetsconney River, coming out of a valley that opens from the north, also empties into the eastern end of North Bentnick arm but north of the mouth of the Bella Coola River.

The water-borne glacial silt of the Bella Coola River is filling in the eastern end of North Bentnick arm, between the mouths of these two rivers, at a rate that has been estimated at about 0.5 foot per year. The tide comes up over the western part of this newly deposited land, which is known locally as the tide flat (fig. 1).

The Bella Coola is a typically U-shaped valley, 1-3 miles wide, hemmed in by mountains that range 4000-10,000 feet high. In some places the floor of the valley has been cleared for farming (fig. 2).

Forests of Bella Coola Valley

WHITFORD and CRAIG (10) state that the valleys that connect the interior plateau with the ocean are a part of the physiographic features of British Columbia that are favorable to economic utilization of forest products. The Bella Coola Valley is one of these favorably located valleys, but up to the present time no widespread cut-



FIG. 1.—Junction of North Bentnick arm and tide flat

tings of the forests have occurred and much of the valley is in its native condition. This made an ecological survey of the vegetation under natural conditions easily possible.

WHITFORD and CRAIG (10) have mapped the five important forest tree species occurring in the Bella Coola region. These are *Pseudotsuga mucronata*,¹ *Thuja plicata*, *Picea sitchensis*, *Tsuga hetero-*

¹ All plants except *Populus tacamahaca* have been named according to HENRY, J. K., Flora of Southern British Columbia. Specimens have been checked with herbarium specimens in the Field Museum, Chicago.



FIG. 2.—Bella Coola Valley looking eastward and showing cultivated areas

phylla, and *Abies amabilis*. The first four make up the climax forest of the valley floor. The last is not found in the valley but is an important montane species. The climax forest does not occur everywhere in the region, but is common on the floor of the western end of the valley. Undergrowth is not common and the forest floor is covered with a thick growth of *Hylocomium splendens*.

In many places in the coniferous forest there are old trees of *Alnus rubra* and *Populus trichocarpa*. These species are not able to reproduce themselves under the conifers, but are pioneer hangovers in the coniferous forest. The percentages of the different species of conifers in the climax forest are somewhat dependent on the location of the forest in the valley. The nearer the forest is to the tide flat the greater will be the percentage of *Picea sitchensis* and the smaller will be the percentage of *Pseudotsuga mucronata*. The closer the forest is to the southern side of the valley the closer it is to the rim of the mountains that hem in the valley, and the later the lie of snow in spring with a greater percentage of *Tsuga heterophylla*. About 7 miles east of North Bentnick arm a careful count showed 32 per cent of *Thuja plicata*, 21 per cent of *Tsuga heterophylla*, 20 per cent of *Pseudotsuga mucronata*, 19 per cent of *Alnus rubra*, and 8 per cent of *Picea sitchensis*. The trees of such a forest are not close together but the shade is dense. The trees are covered with a heavy growth of mosses and lichens.

Pinus contorta is found as a pioneer on thin rocky soil at the eastern end of the valley. The true subclimax of the region is *Pseudotsuga mucronata*. In addition to its occurrence on the mountains, which will be discussed later, this species occurs in almost pure stands on the floor of the eastern end of the valley. It is also important in the mixed forest. The eastern end of the valley is drier than the western end, and therefore favors the growth of this species. However it is not replacing itself, for under the giant trees of *P. mucronata* the undergrowth consists of *Thuja plicata*, *Tsuga heterophylla*, and *Picea sitchensis*. It is evident that on areas that are now covered with *Pseudotsuga* there will be in the future a mixed coniferous climax forest. *Picea sitchensis* forms a pure stand at the back of the tide flat, and extends for half a mile before it merges into the mixed forest of the valley floor.

Tsuga heterophylla forms a large percentage of the mixed forest along the south side of the valley under the shade of the mountains. Both *T. heterophylla* and *T. mertensiana* grow in solid stand in some of the north and south valleys that join the Bella Coola Valley, especially in the Salloompt Valley where the hemlock forests cover the glacial terraces. Hemlocks are common wherever the snow lies until late in the spring.

In some of the north and south valleys *Abies grandis* occurs with the hemlocks. It occupies the moister and more shaded areas. *Tsuga* in pure stand or with a mixture of *Abies* forms a post-climax in the region. The forest of the mountain sides and the succession of alpine vegetation will be discussed later.

Studies of plant succession

I. VEGETATION OF TIDE FLAT

When the swiftly moving Bella Coola River, heavily laden with glacial silt, meets the incoming tide twice each day at the western end of the valley, the current is slowed down and the water spreads out and drops its load. In this way the tide flat is being built up rapidly, and new land is developing for plants to occupy. Many small streams flowing toward North Bentnick arm are visible only during low tide. Drift wood, brought down the river during times of flood, is scattered all over the tide flat. High tide covers the entire western end of the flat, and no woody vegetation occurs.

In all probability the history of the vegetational development of the tide flat is the same as in all parts of the western end of the Bella Coola Valley. It will, therefore, be interesting to follow the development of vegetation on the flat. A careful study of the vegetation was made on a small island south of the main channel of the Bella Coola River.

The greater part of the western end of the tide flat belongs to a vegetation zone which may be called zone 1. It is an area that is covered longest and deepest by high tide. It extends to the east along the little streams. The plants form tiny hummocks, and between these hummocks the water is a few inches deep even during low tide. The plants are rooted in a white, sticky, silty mud. During low tide the water moves slowly over the flat toward North Bentnick

arm, and during high tide it stands over the area to a depth of several feet. The zone is exposed to the fullest light when the tide is out. The area is dominated by *Hippuris tetraphylla* and *Plantago maritima*. In little ponds on the tide flat *Scirpus validus* may be found, but it is not strictly a plant of the first zone. The pH of the zone ranges from 6.6 to 7.0.

Zone 2 is a rather wide area, lying immediately above the preceding. High tide covers it to a depth of 1.5-2 feet. During low tide



FIG. 3.—Zonation of vegetation back of tide flat

it is water soaked and muddy. It has a grassy aspect and is dominated by *Eleocharis palustris*. *Ranunculus cymbalaria* is abundant, and in some parts of the zone *Potentilla anserina* from the zone above is encroaching upon it.

As the ground gets higher it is barely covered by ordinary high tide and is well drained and comparatively dry at low tide. Zone 3 is a marginal band in front of the first zone of woody plants, dominated by *Carex barbarae*, *Potentilla anserina*, and *Trifolium fimbriatum*.

Only exceptionally high tides that are blown in by western storms go higher than zone 3. Above this area on higher land is zone 4, in

the form of a band of woody shrubs dominated by *Myrica gale*, which range in height from 3 to 4 feet. Under the *Myrica* are herbaceous plants such as *Hordeum nodosum*, *Lathyrus palustris*, *Potentilla anserina*, and mosses.

Immediately behind the *Myrica gale* is zone 5. It consists of a narrow band of *Salix lasiandra*, *S. scouleriana*, and *Lonicera involucrata*. These shrubs and small trees range in height from 8 to 10 feet. There is moss on the ground, but the shade is so dense that herbaceous vegetation is not prominent. Young spruce trees are found occasionally under the willows. Behind the willows is an almost pure stand of *Picea sitchensis*. In some places the trees are so close together that other vegetation is almost completely absent and this species is the sole dominant. The spruce zone extends for almost half a mile before it merges into the mixed coniferous forest of the valley floor (fig. 3).

2. SUCCESSION ALONG STREAMS

Since much of the Bella Coola Valley has been made by glacial silt deposited by fresh water, it will be interesting to follow the succession of plants along the streams.

At one place, where 50 m. of new land is known to have been deposited by a stream in the last 25 years, a transect was made so as to extend in a due north and south direction, with the stream at the northern end (fig. 4). When the water of the stream stands at ordinary summer height, a gravelly bank rises to a height of about 0.5 m. above the level of the water. During the periods of summer flood the entire bank is covered with water, and during the fall floods water stands much higher.

Immediately above the bank and paralleling the stream is a belt of young specimens of *Alnus rubra*. This belt is about 3.5 m. wide and extends along the stream for many meters. In places this belt is interrupted. The trees are 1.5–2.5 m. high, with an average height of 2 m. The diameters of the trees are 1–3 cm. The ground on which these trees are growing is somewhat higher than that immediately to the south of it. It is clear that the area occupied by the alders was once a bar, and that the stream flowed at one time through the depression or panne to the south.

The panne is an open area exposed to the full light of the sun. While apparently bare of trees, it is here that the tree seedlings are the thickest. Two species intermingle, *Salix* and *Populus trichocarpa*. No alders are found on any part of the panne (fig. 4). At the

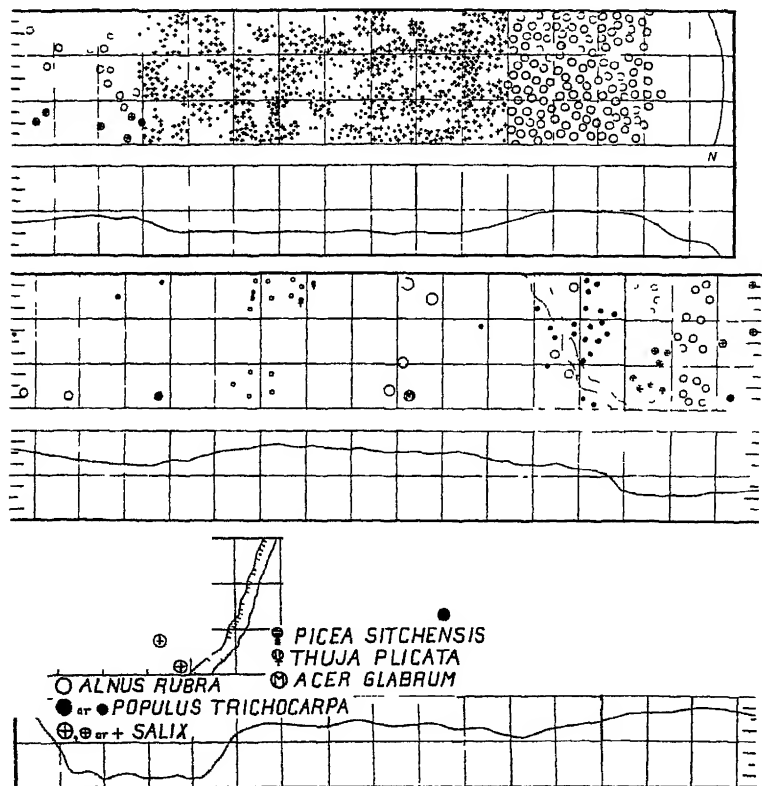


FIG. 4.—Transect across new land made by stream, using 1 m. as unit of measure

present time the panne is beyond the reach of summer floods, but may be covered by the water of fall floods. It is about 15 m. wide, and at the south side the ground rises to a higher level. Near the bank are several small clumps of alders that are about the same size as those along the stream.

The higher land to the south of the panne came into existence as a large island. The trees here are of medium size, scattered, and produce much shade. Many of the largest trees are *Populus tri-*

chocarpa. On the northern side of this land are many medium sized alders. There are a few willows in the depressions, but the trees that are of the most importance from the standpoint of succession are small specimens of *Picea sitchensis* and *Thuja plicata* that are growing in the shade of the larger *Alnus* and *Populus* trees. These indicate that the future vegetation of the area will be a mixed coniferous forest.

At the south side of the area is an old stream bed. The banks on each side of it are rather steep, and on the south bank are old, large alders. In the stream bed are large willows, *Salix lasiandra* and *S. scouleriana*.

It seems proper to draw the conclusion that where new land is formed by stream action, the first trees to grow on areas that are out of the water sufficiently to be well drained are *Alnus rubra*, and on areas that are constantly damp are *Salix* and *Populus trichocarpa*. Moreover, it may be concluded that when sufficient shade is produced to protect the seedlings of *Picea sitchensis* and *Thuja plicata*, these species begin to grow, and if sufficient time is allowed they become the dominant trees of the area. In places that are shaded by the mountains, *Tsuga heterophylla* is found in similar situations.

At the edges of streams which are neither depositing nor cutting to any appreciable degree, shrub vegetation may grow to the very edge. Such shrubs are *Sambucus racemosa*, *Aruncus sylvestris*, *Ribes acerifolium*, *Fatsia horrida*, *Rubus spectabilis*, *Cornus stolonifera*, and *Rubus parviflorus*. With the shrubs are large luxuriant clumps of *Asplenium cyclosorum*. Above the water on well rotted logs one can usually find *Phegopteris phegopteris*. With the shrubs are small coniferous trees. Where coniferous trees have had time to get full grown along a stream that has been neither cutting nor depositing through long periods of years, the climax forest conifers grow down to the water's edge. In many places along the Bella Coola and Nutsatsum rivers the species of *Tsuga*, *Thuja*, and *Picea* grow so close to the edge of the stream that branches hang into the water.

3. SUCCESSIONS IN MUSKEGS

Beavers have played a large part in the ecological development of the Bella Coola Valley. They have built their dams along the

sloughs and across the smaller streams that enter the Bella Coola River, causing the water to spread out and flood the forested areas. Many of the trees have been cut down or killed and the flooded areas have become well lighted. Back from the centers of the flooding the water has spread out, but it has never been deep enough to kill the trees. They have lived on in a less luxuriant way, and there has been produced a water logged area which is well shaded. Thus beavers have produced two new kinds of areas where two distinct types of plant succession may be found. Light is the main factor that determines the differences in these successions: those in the beaver ponds are primary and those in the water logged areas are secondary.

In the deep water of the beaver ponds are submerged algae and mosses. Where the water is more shallow there are pads of *Nymphaea polysepala*, and in still shallower water large areas covered with *Zannichellia palustris* occur. Closer to the shore there is generally a widespread association of *Equisetum fluviatile*. Animals that range the valley, especially cows, keep the *Equisetum* well cropped, and it is not so abundant now as it was when the settlers first came to this region. In the shallower water the *Equisetum* gives way to *Sparganium multipedunculatum*, and shoreward from that two associations of *Carex* succeed each other. *Carex rostrata* grows in deeper water than does *C. salina*. *C. salina* is found more abundantly around the beaver pond at the western end than it is farther east up the valley.

Around the beaver ponds there is usually developed a muskeg where the following plants are found: *Scirpus microcarpus*, *Carex festiva*, *Glyceria pauciflora*, *Sphagnum* sp., and *Polytrichum* sp.

In the moss, either as islands or on the shore of the ponds, there is a well developed shrub association made up of *Myrica gale*, *Spiraea douglasii*, *Lonicera involucrata*, and *Rubus parviflorus* with *Potentilla palustris*. Occasionally, if there is enough shade, *Lysichiton kamtschaticense* may get started. Under the shrubs are seedlings of the deciduous trees and the conifers that originally occupied the area. Almost always the largest seedlings are those of *Alnus rubra* and *Salix*. *S. lasiandra* and *S. scouleriana* are usually both present. The smaller seedlings are those of *Thuja plicata*, *Picea sitchensis*

and *Tsuga heterophylla*. Back of the shrubs is a narrow association of willows and alders in which are growing coniferous trees. It is easy to see that in a few years the flooded area will be covered with the coniferous forest that is native to the region.

Floating logs in the ponds form centers for plant development. It is on these logs that one may always find *Vaccinium canadense*. The logs are usually covered with a thick growth of moss in which are seedlings of *Picea* and *Thuja*. Where the logs are near the edge of the water and are rather dry, there is a good growth of *Cornus canadensis*. Around the shaded margins of the beaver lakes there is a rank growth of *Lysichiton kamtschaticense*. Where the water has never been deep enough to kill the trees, a shade is cast that is sufficient to prevent the growth of light-demanding species. In such places one plant is always dominant, *Lysichiton kamtschaticense*. It comes in where the water is at least a foot deep, and it is still vigorous where there is no standing water and where the ground is firm enough to walk over. In deep water there are algae in addition to the skunk cabbage. *Sphagnum* helps to build a substratum, and on it *Conocephalum* grows.

In order to determine the frequency of the species in the association that had developed on the shaded and water-logged area (8, 4), there were used three thin bars of wood fastened together and arranged so as to fold up and open out. When opened and placed on the ground so as to form three sides of a square meter, the species of plants within the square so formed were noted. By studying a number of sample areas that did not overlap, the percentage of square meters in which each species was found was noted, and these percentages were considered the frequencies of the species in the association.

Such a frequency count gave the following percentages: *Lysichiton kamtschaticense* 96, *Mnium* 60, *Sphagnum* 56, other mosses 48, *Phegopteris dryopteris* 40, *Clintonia uniflora* 40, *Fatsia horrida* 36, *Circaea alpina* 36, *Asplenium cyclosorum* 28, *Hylocomium splendens* 20, *Streptopus amplexifolius* 20, *Alnus rubra* 16, *Equisetum sylvaticum* 12, *Menziesia ferruginea* 12, *Moneses uniflora* 8, *Cornus canadensis* 8, *Picea sitchensis* seedlings 8, *Acer glabrum* seedlings 4, and about four other species in about 4 per cent of all the quadrats ex-

amed. This study shows that *Lysichiton kamtschalcense* and certain mosses are the dominant species in areas that are shaded and water-logged. Where the ground is drier *Fatsia horrida* and the seedlings of the conifers are important.

4. SUCCESSION ON ROCK SLIDES

In the Bella Coola region rock slides are common and are important in the base leveling of the mountains. Where the granodiorite of the mountains approaches granite in structure, the slides are made of huge blocks of rocks; but where the mountains are composed of an easily disintegrated rock, the talus slopes consist of small pieces. On the slides made of the huge blocks of rock, lichens are a much more prominent part of the vegetation than they are on the finer talus slopes. There are four or five species of crustose lichens that are commonly found on the rocks of the Bella Coola region.

In places the crustose lichens may be overgrown by a foliose lichen that grows so close to the rock that it is hard to loosen. Sometimes lichens like *Sticta* follow the crustose lichens, and sometimes *Umbilicaria* comes early in the succession. Frequently *Pilophorus cereolus* follows the smallest lichens, being common on all the rock slides of the region. Where succession has gone further there are many kinds of fruticose lichens, including *Stereocaulon paschale*, *S. prunetosum*, *Ramalina farinacea*, *Thamnolia vermicularis*, *Cladonia gracilis*, *C. bellipodophora*, *C. coccifera*, and perhaps others. In time these fruticose lichens are invaded by *Selaginella rupestris*, *Rhytidium robustum*, *Racomitrium canescens*, *Gymnostomum*, and many other mosses. *Hylocomium splendens* is found in a few of the most shaded and mesophytic parts of rock slides, but it is not at all common.

Rooted in the mosses on the rocks are clumps of *Polypodium vulgare* and *Cryptogramma acrostichoides*. About 25 clumps of *Asplenium trichomanes* were discovered, but it may be more abundant. With it is *Lycopodium lucidulum*. *Campanula rotundifolia* is common. Where soil has accumulated, that is, near the bottom of slides, *Pteris aquilina* var. *lanuginosa* and *Aspidium spinulosum* var. *dilatatum* occur. Where shade is afforded, *Vaccinium parviflorum* occurs; where the mosses have built up a sufficient substratum *Linnaea*

borealis may be found. In the crevices between the rocks of a slide the vegetation makes greater progress, and *Pseudotsuga* comes early. Where shade occurs the seedlings of *Thuja plicata*, *Tsuga heterophylla*, and even a few specimens of *Picea sitchensis* may flourish.

On one of the slides at the western end of the valley on a western exposure is a dense growth of *Gaultheria shallon*. It grows in the shade of trees and other shrubs, and is found only on the western exposure of one mountain in the Bella Coola region, although it is common along the channels, especially near Ocean Falls. With this may be found *Vaccinium parviflorum* and *Spiraea discolor*.

About 1885, on South Bentnick arm near a mine owned by Mr. F. Jacobsen, there occurred a big rock slide. Mr. Jacobsen said that 40 years later the lower part of the slide was completely grown up to *Thuja plicata*, *Tsuga heterophylla*, *Picea sitchensis*, and *Alnus rubra*, and that the trees were 12-14 inches in diameter.

5. SUCCESSION ON CUT-OVER LAND

Clearing in the Bella Coola Valley may be complete or partial, and the degree of clearing is important in determining the type of secondary growth. At the back of the tide flat the spruce forest has been cleared in places to allow grazing space for animals. In some places *Trifolium repens* and *T. fimbriatum* cover the ground. *Agrostis alba* is found with *Trifolium* in some places. Other herbaceous plants in the cleared areas are *Lilium parviflorum*, *Sanguisorba sitchensis*, *Prenanthes alata*, *Trientalis arctica*, *Potentilla anserina*, *Asplenium cyclosorum*. Some parts of the area are becoming overgrown with shrubs, *Rosa nutkana*, *Amelanchier florida*, *Spiraea douglasii*, and *Lonicera involucrata*. Under these shrubs there are seedlings of *Picea sitchensis*. Clumps of half-grown *Picea* are found on all parts of the cleared area at the back of the tide flat.

Where the land was covered with a mixed conifer forest, before it had been cleared for use as pasture land, the plants of the secondary growth represent three general groups, those of the former forest, those of the pasture, and those of the new forest that is establishing itself.

A count on one pasture that is reverting to brush showed that *Rubus viburnifolia* has a frequency of 90, *Poa pratensis* 80, *Populus*

tacamahaca 70, *Trifolium repens* 60, *Cornus stolonifera* 40, *Rubus parviflorus* 35, *Alnus rubra* seedlings 30, *Galium triflorum* 50, *Cerasium vulgatum* 25, *Rumex acetosella* 25, *Sambucus racemosa* 25, *Phegopteris dryopteris* 20, *Ribes divaricatum* 15, *Urtica* 15, *Asplenium cyclosorum* 15, *Spiraea douglasii* 10, *Plantago major* 10, *Picea sitchensis* seedlings 10, *Thuja plicata* 5, *Salix* 15, *Montia sibirica* 5, and *Maianthemum bifolium* var. *kamtschaticum* 5.

The plants of the former forest are in the minority, and are represented by such plants as *Montia sibirica*, *Maianthemum bifolium* var. *kamtschaticum*, and *Phegopteris dryopteris*. The pasture plants are represented by *Poa pratensis* and *Trifolium repens*. The plants which represent the new forest which is in process of becoming established are *Rubus viburnifolia*, *R. parviflorus*, *Populus tacamahaca*, and seedlings of *Alnus rubra*, *Picea sitchensis*, and *Thuja plicata*.

The first plant to invade a pasture which is not being used sufficiently is *Rubus viburnifolia*. Then the seedlings of *Populus tacamahaca* get started and grow into tall trees. In the shade of the deciduous vegetation soon come the seedlings of the conifers *Tsuga*, *Picea*, and *Thuja*. In some places the poplars are 30 feet tall, *Alnus* almost as tall, and the conifers under them about 5 feet high. All this has come on land that was cleared less than 30 years ago, indicating that land becomes reforested rapidly. When the trees have not been cut down in sufficient numbers to clear the ground, and where the partially cleared ground is well shaded, the conifer seedlings that are native to the soil soon replace the trees that were cut.

At the back of the tide flat *Picea sitchensis* seedlings follow on the partially cleared land. Farther east *Picea sitchensis*, *Thuja plicata*, *Tsuga heterophylla*, together with such shrubs as *Sambucus racemosa* soon follow the cutting of the timber. Far up the valley, where the forest is partly cut, seedlings of *Pseudotsuga mucronata* cover the cleared land. In the side valleys, where there is a pure stand of hemlock (*Tsuga heterophylla* and *T. mertensiana*), seedlings of these follow the partial cutting of the timber.

6. VEGETATION ON BURNED AREAS

About 30 years ago there was a severe fire in the Bella Coola region. Since that time there have been no fires of any extent in the

valley, and therefore there was no chance to study the successions that follow recent burns; but present conditions of areas that were burned 30 years ago were studied.

On the mountain side that faces south the burned areas are grown up to an almost pure stand of *Pseudotsuga mucronata*. Where the ground is rocky *Pseudotsuga* is replaced by *Pinus contorta*. The trees are so crowded that some of them are dead, and the shade is so dense that there is little or no vegetation under the trees. The scattered growth consists of *Aralia nudicaulis*, *Arctostaphylos uva-ursi*, *Pyrola minor*, *P. asarifolia*, *Rubus parviflorus*, and a few trees of *Alnus rubra* and *Acer glabrum*.

Where the burned area has a west exposure, under the *Pseudotsuga* occur *Rubus parviflorus* and seedlings of *Tsuga*, *Picea*, and *Thuja*. Conditions on the western exposure are much more mesophytic than on the southern exposure. Where burned areas face north, conditions are very different. Large areas are covered with *Alnus sitchensis*, *Amelanchier alnifolia*, and *Acer glabrum*. Under the deciduous trees are seedlings of *Picea*, *Tsuga*, and *Thuja*. Sometimes both *Tsuga heterophylla* and *T. mertensiana* are found. The deciduous vegetation is much more marked on the north facing mountain than it is on the south facing slopes. At the eastern end of the valley, on a small recently burned area, small *Pseudotsuga* trees cover the ground. On rocky places *Pinus contorta* replaces the *Pseudotsuga*.

Vegetation on eroding surfaces

There are two types of eroding surfaces in the Bella Coola region, glacial terraces in the side valleys that open into the Bella Coola Valley and river terraces along the sloughs through which the flood waters of the Bella Coola River flow during times of flood.

Many of the side valleys were preglacial and filled with gravel at the end of the ice age. Since that time mountain streams have been eroding them, in some places the erosion still occurring. Trees of *Tsuga* are found sliding down such banks, and they grow poorly. The only pioneer tree on such steep slopes is *Alnus rubra*. Such banks are open and exposed to erosion. Along the sloughs the flood waters have cut as many as two or three terraces. *Alnus rubra* constitutes 80 per cent of the tree growth, and *Salix scouleriana* and *S.*

lasianra 20 per cent. These trees are the pioneers, and under them come *Sambucus racemosa*, *Lonicera involucrata*, *Rubus parviflorus*, and *Symphoricarpus racemosa*. Herbs are not common, *Erigeron philadelphicus* and *Montia sibirica* and in shadier places *Circaea alpina* being found most frequently. *Acer glabrum* and seedlings of *Thuja plicata* and *Picea sitchensis* are found under the willows and alders on the banks.

Mountain vegetation

A detailed study of the vegetation of the mountains of the region and a study of the succession of vegetation on Canoe Crossing Mountain were made. Canoe Crossing Mountain is a part of the northern rim about 20 miles up the valley. It is not a high mountain, being about 1829 m. above sea level. Because of the latitude this is well above the timber line, and affords an excellent study of the alpine and subalpine regions. The vegetation at the top will be discussed first, because all of the vegetation of the mountain has developed since the melting of the glacial ice. The top of Canoe Crossing Mountain has been thoroughly glaciated, as is shown by the glacial grooves, glacial striae, volcanic and other erratics. The glacial grooves show that the valley glacier rode up over the end of the mountain.

1. SNOW FIELDS

Near the top of the mountain, especially in sheltered places on the northern and eastern exposures, there are large banks of perpetual snow. This snow is dimpled from exposure to wind and sun. Red snow (*Chlamydomonas nivalis*) is common on these snow fields. This alga extends deeply into the snow and is better developed in the hollows than on the little ridges between the depressions. The snow fields with northern exposure are thoroughly red with the algal growth. The amount of *Chlamydomonas* on the snow with eastern exposure is still less. On snow fields facing west and south there is little or none.

2. SUCCESSION IN ALPINE REGION

BED ROCK.—At the top of the mountain there is much bed rock that is exposed to the action of light, wind, and cold. The important crevice plant is *Silene acaulis*. If bed rock is more or less vertical

and well drained, three crustose lichens are the pioneers. They are *Rhizocarpon geographicum*, *Acarospora* sp., and an unidentified lichen. The crustose lichens may be overgrown by a small, black fruticose lichen, *Alectoria divergens*, or by a larger black foliose lichen, *Gyrophora erosa*. Sometimes the latter follows *Alectoria*. On the most exposed parts of the bed rock there is no further stage in the succession.

Where bed rock has been hollowed out by ice, and snow lies until late in the spring, the pioneer is a dark colored moss. *Saxifraga tolmiei* starts in the moss if it is sufficiently wet. Where the moss dries out early in the season *Crocynia* sp., a sorediose lichen, may cover the moss. *S. tolmiei* is followed by *Carex tolmiei*, and it in turn is followed by *Luzula parviflora*, *Juncus subtriflorus*, *Poa alpina*, *Phleum alpinum*, *Trisetum spicatum*, and *Veronica alpina*. In most cases the next association is one of *Spiraea pectinata*, which is followed by *Phyllodoce glandulosa* and *Cassiope mertensiana*. Then follows the climax of the alpine region, *Phyllodoce empetriformis*. With this is *Lycopodium sitchense*. In some of the basin areas the succession goes no further than *Cassiope mertensiana*.

Where the alpine region, which is characterized by the absence of upright trees, merges into the subalpine region with its dwarfed but upright trees, the heather on rocky areas which are exposed to the wind is followed by a poor growth of wind timber made up of *Abies lasiocarpa*, *Pinus albicaulis*, and *Juniperus communis*.

TALUS SLOPES.—Most of the mountain is made up of talus. There may be streams, or the water may spread out into shallow pools. In some places the talus is dry throughout the growing season and in other places it is always wet. Along streams that are deep with rather rocky banks are two pioneers, *Caltha leptosepala* and *Trollius laxus*. *Mimulus alpinus* and *Parnassia fimbriata* sometimes grow with them. On higher ground is *Spiraea pectinata*, which in turn is succeeded by the three heathers with *Phyllodoce empetriformis* and *Lycopodium sitchense* as the climax.

Where the running water spreads out into a thin sheet over the talus, mats of moss fill up the water. *Romanzoffia sitchensis* grows in such wet places. Where the moss is not so boggy *Saxifraga tolmiei* occurs. Boggy places in the talus usually have a dense growth of

vinelike willows, *Salix arctica* and *S. barclayi*, around the edge. Back from such places is the usual succession of *Spiraea pectinata* and the three heathers

Where the talus slope becomes dry in the early part of the growing season certain mosses are the pioneers. These are followed by a growth of lichens, of which *Crocynia* sp., *Cetraria islandica*, and *Cladonia pyxidata* are the most prominent. Sometimes *Cladonia rangiferina* is found also. *Solorina arctica* and another large green foliose lichen soon overwhelm the smaller lichens and the moss. Usually the series is ended by a dense growth of fruticose lichens, among which may be mentioned *Stereocaulon tomentosum* and a *Cladonia*.

The mosses and the lichens are followed by flowering plants, such as *Saxifraga tolmiei* and *Gentiana propinqua*. In some places the following make a colorful mat, rooted in the moss: *Sibbaldia procumbens*, *Saxifraga bronchialis*, *Pentstemon confertus*, *Pedicularis scopulorum*, *Senecio pauciflorus*, *Oxyria digyna*, *Saxifraga lyallii*, *Pedicularis scopulorum*, *P. bracteosa*, and many others. Often *Campanula rotundifolia* grows in the talus around imbedded large rocks. *Silene acaulis* may sometimes be a pioneer between pieces of talus. Behind the herbaceous associations there always comes *Spiraea pectinata*, followed by the three heathers with *Phyllodoce empetri-formis* as the climax.

Some parts of the talus are kept wet throughout the summer by the melting snow. The water is below the surface of the talus and is moving downward all the time. On such places is the best development of the lupine fields. In the wettest places are quantities of *Trollius* also. Several species of *Castilleja* occur, and where it is very wet there may be found creeping willows. Back from such places, where the talus is better drained, occurs the regular succession of *Spiraea pectinata* followed by *Phyllodoce glandulosa*, *Cassiope mertensiana*, and *P. empetri-formis*. With the heather there may be *Lycopodium sitchense*.

The pioneers that come in the alpine region depend upon the amount of water and upon the condition of the rocks, but in all parts of the alpine region on Canoe Crossing Mountain, every sere ends with *Spiraea pectinata*, followed by *Phyllodoce glandulosa*, which in

turn is followed by *Cassiope mertensiana* and the climax species *Phyllodoce empetriformis*.

3. SUCCESSION IN SUBALPINE OR HUDSONIAN ZONE

At an altitude of about 1585 m. the alpine region merges into the subalpine or Hudsonian zone. This zone extends down the mountain to about 1463 m. Altitudinally it covers about 122 m. On Canoe Crossing Mountain the subalpine zone covers considerable area because the mountain at this altitude is rolling and not at all steep. It has a parklike appearance, with scattered clumps of trees and much low heather. Where the alpine region merges into the subalpine there is a good growth of wind timber, consisting largely of *Abies lasiocarpa* and *Juniperus communis*. Somewhat lower down the trees are upright, but have no branches on the windward side.

Some of the lakes in the subalpine area are deep, with rock bottoms. Around such lakes is *Spiraea pectinata*. In the upper part of the subalpine the *Spiraea* is followed by *Phyllodoce glandulosa*, but in the lower part this is absent and the *Spiraea* is followed by *Cassiope mertensiana*. *Phyllodoce empetriformis* follows *Cassiope*. In the upper part of the zone this species gives way to clumps of trees, mainly *Abies lasiocarpa*, together with a few *Pinus albicaulis*. However, in most of the subalpine area *Phyllodoce empetriformis* is followed by *Rhododendron albiflorum*, *Vaccinium caespitosum*, and *Veratrum viride*. This shrub association is followed by tree growth.

Shallow lakes usually fill up with mats of moss which appear to be rather solid, but which are not safe to walk upon. In this moss, *Caltha leptosepala* and *Trollius laxus* are usually found. *Salix arctica*, *S. barclayi*, and *S. barrattiana* grow around these ponds. They are usually taller on the west sides of such lakes than they are on the east, because the subalpine zone of Canoe Crossing has a western and southern exposure, and the snow on the western side may melt earlier than the snow on the eastern sides. The willows may be vine-like on the east side of such a lake and several feet high on the west side. Around such lakes the succession goes from *Spiraea pectinata* to trees in the order already discussed.

Sometimes the streams that flow through the subalpine region are slow and sluggish. Along such streams are quantities of moss in

which *Caltha* and *Trollius* grow. With them *Saxifraga tolmiei* may be found. Beyond the edges of such boggy places is *Spiraea pectinata*, followed in the regular way by the heathers and *Rhododendron*. In the lowest part of the subalpine area *Pyrus occidentalis* may grow with the *Rhododendron*. These shrubs and small trees are followed by *Abies lasiocarpa*. When streams flow through rocky channels, the pioneers are *Trollius laxus* and *Caltha leptosepala*. *Phyllodoce empetriformis* occupies a large area in this region.

Sometimes the streams are shallow and spread over rocky areas. Here are produced the beautiful wet meadows similar to those in the alpine zone. *Caltha* and *Trollius* are often in the wettest places. Where there is less moisture there are clumps of *Lupinus arcticus* and *Castilleja*. The lupines are succeeded by the heathers and *Veratrum viride*. Where the ground is perfectly level the lupines are often lacking.

In subalpine ravines there is a marked difference on the west facing and east facing sides. The east exposure may be in the condition of the alpine and the west exposure be so thickly covered by alpine firs as to suggest the Canadian or montane zone. The difference is due to the fact that the snow melts more rapidly on the west slope in the early summer. All the firs in the subalpine region, as well as those lower down, are overgrown by *Alectoria fremontii*. In many places there are rocky ledges exposed to the wind where the wind timber grows and the vegetation is alpine rather than subalpine.

4. VEGETATION IN MONTANE ZONE

PIPER (7) states that the Canadian zone is the most poorly defined zone in the state of Washington. The most characteristic tree in Washington is *Pinus monticola*. *Abies amabilis* and *Tsuga heterophylla*, and sometimes *Thuja plicata*, are also found in the Canadian zone of Washington. On Canoe Crossing Mountain the montane zone extends from about 853 m. to about 1463 m. (fig. 5). The montane zone is made up of three great bands of forests, each occurring as an almost pure stand of a single species. The uppermost band of trees is a forest of *Abies lasiocarpa* with a few scattered specimens of *Pinus albicaulis* and *Tsuga mertensiana*.

Around Mud Lake, which is in the upper part of the montane,

the succession of vegetation is as follows. The open water of the lake is full of a brown floating moss (*Amblystegium*). The first zone

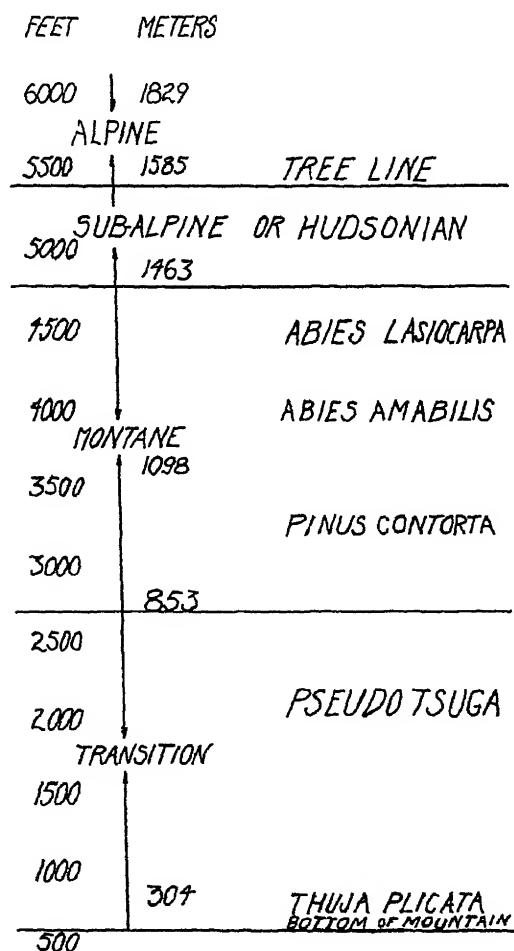


FIG 5.—Profile of Canoe Crossing Mountain showing dominant vegetation at different altitudes.

of plants rooted in the mud beneath rather deep water is *Carex rostrata*. Closer to the shore is *Eriophorum polystachion*. Shoreward from that is a close growth of *Sphagnum* and other mosses in which

is growing *Carex decidua*. An association of *Carex phaeocephala* follows this. With it is *Poa alpina*.

Out of the reach of water during most of the growing season is a wide and well developed zone extending entirely around the lake, made up of *Spiraea pectinata*, *Luzula divaricata*, *Veratrum viride*, *Valeriana sitchensis*, *Deschampsia atropurpurea*, and several composites.

The two heathers, *Phyllodoce glandulosa* and *Cassiope mertensiana*, which usually follows *Spiraea* in the alpine and subalpine regions, have been eliminated and *Spiraea pectinata* is followed by *Phyllodoce empetrifomis* in the upper part of the montane zone. *Phyllodoce empetrifomis* was not found below 1310 m. Around Mud Lake the heather is followed by *Rhododendron albiflorum*, *Pyrus occidentalis*, *Veratrum viride*, and *Lupinus arcticus*. This association is succeeded by *Abies lasiocarpa*, growing so densely that there is little or no undergrowth. At about 1310 m. the band of alpine firs gradually merges with a forest of *A. amabilis*. This species forms an almost solid stand down to 1098 m. The lower part of the forest gradually merges into a forest of *Pinus contorta*, which extends from 1098 to 853 m. Most of the trees are 0.3 m. in diameter and are tall and well developed. The pine forest gradually merges into the transition zone below.

5. TRANSITION ZONE

On Canoe Crossing Mountain much of the soil is made up of small talus material. The slope, although steep, is remarkably free from gullies. There are a few areas of bed rock, but on the mountains west of this elevation are many large areas of exposed rock. There are a few canyons on the mountain side. The lower 200 feet of the mountains that face the south are mesophytic.

Canoe Crossing Mountain begins to rise from the Bella Coola Valley at about 244 m. above sea level. From about 305 m. above sea level the mountain is dominated by *Pseudotsuga*, except on rocky areas. Some of the trees are giants and others are second growth. The lower part of this forest is open and easy to traverse. There is a scattered growth of *Pachystima myrsinites*, *Arctostaphylos uva-ursi*, *Epilobium angustifolium*, *Spiraea discolor*, *Shepherdia canadensis*, and a few other species. The north sides of the trees,

but never the south sides, are covered with a long yellow fruticose lichen. Above 487 m. the undergrowth is much denser, and consists of the preceding species, together with *Pteris aquilina*, *Rubus parviflorus*, and *Cornus canadensis*. Where the shade is thick *Polypodium vulgare*, *Pyrola* sp., and other species occur. Deciduous trees are more in evidence, and *Alnus tenuifolia*, *Amelanchier alnifolia*, and *Acer glabrum* are found. In the more open parts of the forest are young trees of *Pseudotsuga*. In shady parts the undergrowth consists of young trees of *Thuja plicata* and *Tsuga heterophylla*. These species seem to be succeeding *Pseudotsuga*, especially where there is sufficient shade. Often the benches on the sides of the mountain are covered with a very mesophytic forest.

Much of the slope of the south facing mountains is made up of bed rock. Such areas are covered with many kinds of mosses, including *Gymnostomum* sp., *Rhytidium robustum*, *Racomitrium canescens*, and *Mnium insigne*. Almost never does one find *Hylocomium splendens* on the south facing slopes, although it is common on the floor of the valley.

In the mossy areas are patches of *Peltigera*, *Cladonia*, and *Selaginella rupestris*. In summer the patches of mosses have a red brown color and may be seen at great distances. *Cryptogramma acrostichoides* and *Polypodium vulgare*, *Campanula rotundifolia*, *Sedum*, and *Epilobium angustifolium* are also found on the moss covered rocks.

Some of the rocks are covered with shrubs, such as *Pachystima myrsinites*, *Menziesia ferruginea*, *Amelanchier alnifolia*, and *Acer glabrum*. *Pinus contorta* occurs on these rocky areas. Its roots are shallow and able to spread out over the rocks.

Near the bottom of the mountains the forest is very mesophytic. *Pseudotsuga* is mixed with *Thuja plicata*, *Picea sitchensis*, *Alnus rubra*, *Populus trichocarpa*, and *Acer glabrum*. The shrubs of this forest are *Cornus stolonifera*, *Physocarpus*, and *Spiraea discolor*.

The ravines and canyons on the mountain sides are filled with *Fatsia horrida*, *Menziesia ferruginea*, and *Vaccinium ovalifolium*. The tree growth is dense, and consists of *Thuja plicata* and *Acer glabrum*.

6. NORTH FACING MOUNTAINS

The mountains that border the south side of the Bella Coola Valley are much more dissected by small streams and little valleys than are the mountains along the north side of the valley; therefore there are more west, northwest, east, and northeast facing slopes than there are due north exposures. These variations are significant, and are quickly reflected in the species of conifers that are dominant on any particular area.

The west exposure of the lower part of the mountain is covered with a mesophytic forest in which *Thuja plicata* makes up about 35 per cent of the trees, *Tsuga heterophylla* and *T. mertensiana* 20 per cent, *Picea sitchensis* 18 per cent, *Alnus rubra* 10 per cent, *Pseudotsuga mucronata* 8 per cent, *Betula occidentalis* 5 per cent, *Acer glabrum* 3 per cent, and *Populus trichocarpa* 1 per cent. The specimens of *Picea*, *Thuja* and *Tsuga* are large too, but they are not so gigantic as *Pseudotsuga*.

There is dense shade in such a forest and the ground is thickly covered with *Mnium* and *Hylocomium*. The ferns are, *Phegopteris dryopteris*, *Asplenium cyclosorum*, *Aspidium spinulosum*, and *Polystichum munitum*. Such shrubs as *Viburnum pauciflorum*, *Rubus parviflorus*, and *Aruncus sylvestris*, and such herbs as *Actaea eburnea*, *Chimaphila umbellata*, and several species of *Pyrola* form a scattered growth.

Well up on one of these mountain slopes with a western exposure is a bench along a mountain stream where there is growing a small group of *Populus tremuloides*. This is the only place in the whole region where this species is known to occur.

Where the mountain faces due north, the forest is made up almost entirely of *Tsuga heterophylla* and *T. mertensiana*. The ground under these trees is completely covered with many kinds of lichens and moss. The most prominent of these are *Peltigera*, *Cladonia*, and *Hylocomium*. Carvings made by the Indians on big boulders and bed rock have been entirely covered with the moss and lichen growth.

On the lower part of the mountain, where large areas of bed rock are exposed, especially where the surface of the rock slopes steeply, *Pinus contorta* is the edaphic dominant. Where the mountain slope

has an east exposure, especially when the side valleys are narrow and the slope is protected from the sun, the forest consists of *Tsuga* intermingled with *Abies grandis*. In such situations the moss is very thick. The snow lies deeply on the ground in the winter and remains until late in the spring. Much moisture is retained in the moss from the snow, rains, and fogs. The *Tsuga-Abies* forest of these east slopes may be considered a post-climax in the region.

No study of the upper slopes of the north facing mountain was made, but residents of the valley who have been on top of these mountains say that the alpine region is covered with the same kinds of heather as are found on the south facing mountains.

Wherever the north facing mountains were burned, some 30 years ago, the slopes have grown up to deciduous vegetation consisting of *Acer glabrum* and *Alnus sitchensis*. Under the trees are halfgrown trees of *Picea sitchensis*, *Thuja plicata*, *Tsuga heterophylla*, and *T. mertensiana*. Such a forest is difficult to traverse, and contrasts strongly with the open *Pseudotsuga* forest on the south facing slopes, which has also grown up since the fire 30 years ago.

Side valleys

The large side valleys that open into the Bella Coola Valley extend in a north and south direction. It is evident that some of them have been preglacial, because they are filled with glacial terraces. Salloompt Valley opens from the north, and its southern end is wide for at least 2.5 miles north of the Bella Coola River. Then the valley becomes narrower and the glacial terraces become prominent. Deep snow lies in the valley in winter, but in early spring the snow melts sooner than it does in the Bella Coola Valley, and for this reason the vegetation of the south end of Salloompt Valley is much more advanced in the spring than vegetation of the Bella Coola Valley.

At the southern end of Salloompt Valley are areas that are grown up to an almost pure stand of *Pseudotsuga*. Farther to the north the forest is more mixed. Large trees of *Picea sitchensis*, *Thuja plicata*, and *Tsuga heterophylla* make up the forest. The undergrowth consists largely of *Tsuga*. Apparently the *Tsuga* forest is a post-climax for the region.

On the terraces the forest consists of *Tsuga* 64 per cent, *Pseudo-*

tsuga 17 per cent, *Thuja plicata* 10 per cent, *Pinus contorta* 2 per cent, *Betula* 1 per cent, *Picea* 4 per cent, and *Alnus rubra* 2 per cent. Openings in the forest are covered with *Pteris aquilina* var. *lanuginosa*. Most such forests have little undergrowth except *Hylocomium*. What there is, is scattered and consists of *Linnaea borealis*, several species of *Vaccinium*, and several kinds of *Pyrola*.

The valleys that open into the Bella Coola Valley from the south retain the snow until late in the spring, and the vegetation is apt to develop later than does vegetation in the Bella Coola Valley. The forests in these shaded and very mesophytic valleys consist largely of *Tsuga heterophylla* and *T. mertensiana*, together with *Abies grandis*. All north and south valleys are characterized by the absence of *Populus trichocarpa*. *Fatsia horrida* is common close to the streams, and in openings.

Floristics

When the continent of North America was cut into two parts by the great Rocky Mountain geosyncline in the Cretaceous period, western North America was a peninsula of Asia, and the eastern part of the continent was attached to Europe. HARSHBERGER (5) states that it was at that time that the eastern and western conifers were assorted and established as distinctly eastern and western species. In the Bella Coola region there is only one species of conifer that is found in the eastern part of the continent. This is *Juniperus communis*, and it is found only in the alpine and subalpine zones, where it occurs as wind timber.

PIPER (7) says that the facts in the distribution of *Picea sitchensis* are peculiar. It is the dominant tree, forming more than 50 per cent of the forest strip along the coast from middle Oregon northward to Kodiak Island, beyond which all timber ceases and the flora becomes that of the Arctic zone. In the north this species ends sharply in the Arctic meadows, and in the south it merges gradually with the *Pseudotsuga* forests. This enormous stretch of a single species at sea level is probably due to the remarkably equable temperature and great humidity of the immediate sea coast. According to WHITFORD and CRAIG (10), *Picea sitchensis* is confined to the Pacific Coast, seldom extending back farther than 50 miles from the salt water. It seldom occurs in a pure stand, and is a prolific seeder.

Abies grandis occurs rarely in the Bella Coola region. It is found in the side valleys that open into the Bella Coola Valley from the south. These valleys are closely shaded by the mountains and open to the north, and they are deeply filled with snow until late in the spring. *Abies grandis* does not occur in a pure stand, but is always found growing with *Tsuga heterophylla* and *T. mertensiana*. Mr. F. Jacobsen, who lives in the valley, says that *Chamaecyparis nootkatensis* is found in only one place in the Bella Coola region, and that is far up in one of the mountains along the northern border at the western end of the valley.

There are only two pines found in the area studied. *Pinus albi-caulis* grows in the alpine and subalpine zones but not abundantly. *P. contorta* grows as an edaphic pioneer on rocky places, and is found abundantly in the montane zone. *Pseudotsuga mucronata* evidently has come into the Bella Coola Valley from the interior plateau, and is found at its best at the eastern end, where the climate is more continental than it is nearer the coast.

Both *Tsuga heterophylla* and *T. mertensiana* grow together in the valley and on the north facing slopes. In the valley bottom *T. heterophylla* is much the commoner of the two in the mixed forest.

The poplars of the region are interesting. *Populus trichocarpa* is the common species. It is a pioneer on damp ground along streams, and is almost the ecological equivalent of the willows in the seedling condition, but in the climax forest the poplars are present as hangovers because they are long-lived trees. *P. trichocarpa* is seldom or never found in any of the north and south valleys. For its establishment as a seedling it needs strong light as well as moisture. Sufficient light is not available in north and south valleys. *Populus tacamahaca* is found rarely in the valley. It is a pioneer on cutover land that has been pastured. *P. tremuloides* is found in but one place in the Bella Coola region. It grows on one of the mountains that borders the south side of the valley, along a mountain stream.

There are two willows in the valley and three in the alpine and subalpine parts of the mountains. The two species of low altitude are *Salix lasiandra* and *S. scouleriana*. The three species of the mountain tops are *S. arctica*, *S. barrattiana*, and *S. barclayi*.

The ferns of the region are cosmopolitan. *Asplenium trichomanes* grows in about 25 clumps on the large rock slide at the western end of the valley. Only this station was found for it. *Botrychium simplex* and *B. ramosum* were found in one location, but since they are so small they may have been overlooked in other places.

Many species of heaths are common, but the distribution of *Gaultheria shallon* is the most outstanding of all. It is found in only one place in the region. It grows on the west exposure of a mountain at the western end of the valley along the northern rim. Here it occurs in the shade of the coniferous forest, and makes traveling through the forest difficult. This very local growth, as contrasted with its common and widespread occurrence along the channels farther west, is noteworthy.

Lichens are abundant everywhere. They cover the fence posts, tree trunks, tree limbs, and rocks from sea level to the mountain tops. No *Fucus* is found in the waters of North Bentnick arm near the mouth of the Bella Coola River. This species is common along the coast, and its absence here is noteworthy and is probably due to the dilution of the saline waters of North Bentnick by the fresh water of the Bella Coola River.

This work was done under the direction of Dr. HENRY C. COWLES, and to him I wish to express thanks for advice and for the loan of certain instruments. I am also indebted to Dr. GEORGE D. FULLER for much valuable help and for the loan of instruments, to Dr. BRUCE FINK and to Dr. CHARLES PLITT for identifying the lichens, to Dr. JOHN DAVIDSON for identifying certain of the herbaceous species, and to Miss MILDRED IRWIN for identifying certain of the mosses.

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[Accepted for publication January 6, 1931]

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GERMINATION OF SPORES AND DEVELOPMENT OF JUVENILE THALLUS OF *RIELLA AMERICANA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 420

R. A. STUDHALTER

(WITH TWENTY-FIVE FIGURES)

Introduction

Botanists have long been interested in *Riella*, a genus of submerged aquatic liverworts, because of its peculiar shape. The adult plant consists of a thickened stem, on one side of and over the tip of which is a wing one cell in thickness. Rhizoids develop near the base of the stem; archegonia are produced along its margin; and antheridia develop along the outer edge of the wing, which is here somewhat thickened. The origin and morphological interpretation of the wing have been subjects of much controversy.

The first known species of *Riella* was called *Sphaerocarpus notarisii*, and was described from Sardinia in 1839. With the discovery of a new form in Algeria, the two species were placed together into the new genus *Duriæa* in 1843. In 1852, MONTAGNE added a third species from Switzerland to the group, and changed the name to *Riella* on account of the previous use of the name *Duriæa* for a genus of the Umbelliferae. Eleven species are now known, from Algeria, Sardinia and Greece, Switzerland, France, Turkestan, South Africa, the Canary Islands, and Texas. The distribution of each is more or less local, some having been collected only a few times; and one species has not been seen in its native habitat, having been seen only in culture from mud collected in Turkestan (11).

Riella americana was described in 1903 by HOWE and UNDERWOOD (9) from specimens collected in western Texas. This, together with a fragmentary specimen from the same place in 1855, and another fragmentary specimen from South Dakota in 1898, are the only recorded collections of the species until its rediscovery by the writer

in the spring of 1927. The work of HOWE and UNDERWOOD is apparently the only published paper on the species.

Materials and methods

The spores used in the germination studies herein reported were obtained in western Texas at various times from 1928 to 1930. Plants with mature sporophytes were collected, together with some of the sand, from the bottom of the creek in which they were growing. In most instances this mixture was permitted to air-dry in the laboratory, but in other cases it was kept in water in a closed jar until used.

For purposes of germination, some of this material was placed during the warmer months of the year in a moist chamber or jar in 2-3 inches of distilled water and placed on window sills where direct sunlight was available part of the day. In other instances the cultures were heated to temperatures ranging from 40° to 75° C. for one or two hours before being placed in the window. The evaporated water was replaced with distilled water, and occasionally a mixture of distilled and tap water. The presence of the sand from the natural habitat is believed to have furnished a partially natural condition for the material.

For the purpose of study, some of the sediment in the bottom of the culture was drawn off with a wide mouthed pipette. Every few days a portion of the material was preserved in formalin-alcohol for future comparison with the fresh green sporelings, the latter serving as the basis for most of the study as well as for the drawings.

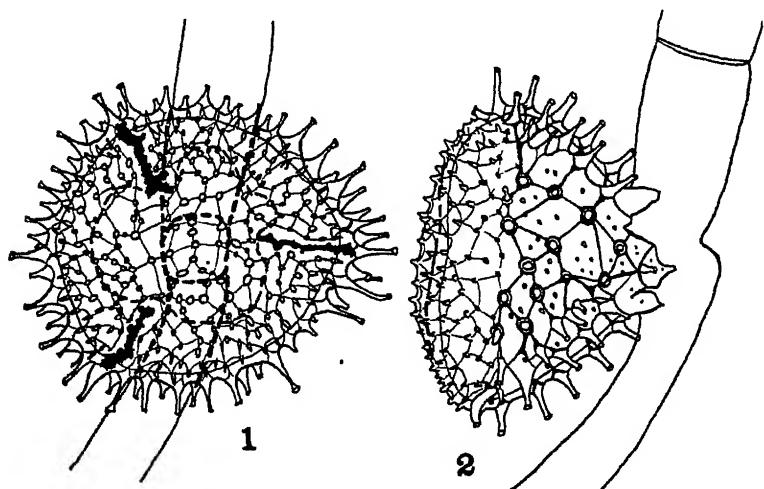
The more elaborate methods used by other workers in germinating the spores of *Riella*, often in addition to simple water cultures, were not found necessary for *R. americana*. PORSILD (12) used mud, filter paper, loess, or kaolin covered with water. HOWE and UNDERWOOD (9) used filter paper. GOEBEL (6) also used kaolin. It is believed that pipetting the sporelings is less injurious to them than picking them off of a more solid substrate; but this method has the disadvantage of making it more difficult to obtain a clear idea of the orientation of the sporelings in the water, and of disturbing this orientation more frequently.

First germination stages

The spores of *Riella americana* are described by HOWE and UNDERWOOD (9) as follows:

dark-brown, 100-130 μ in maximum diameter (spines included); outer face bearing numerous sometimes curved spines 10-24 μ long, with dilated apices, these spines more or less connected by radiating basal membranes forming irregular reticulations; inner faces bearing conical, non-capitate spines, 3-6 μ long, with basal membranes obsolescent or entirely wanting.

As the spore matures, the outer face becomes somewhat conical and the inner face flattened or even depressed at its middle. The



FIGS. 1, 2.—Fig. 1, germinating spore from inner face; long capitate spines of outer face extend over margin; irregular radial ridges shown in solid black, boundary of central depression dotted; fig. 2, germinating spore from side; germ tube emerging from middle of outer and more spiny face; $\times 420$.

triradial ridges are usually completely obliterated, and, if seen at all, are merely rough, irregular, dark ridges toward the outer portion of the inner face (figs. 1, 2).

The spores of *R. americana* are found to germinate better after a period of rest. Fresh spores, apparently mature, give a low percentage of germination; while spores from the same material up to 17 months of age (the oldest available) which had been kept dry or in water in the dark, germinated in excess of 50 per cent in a few

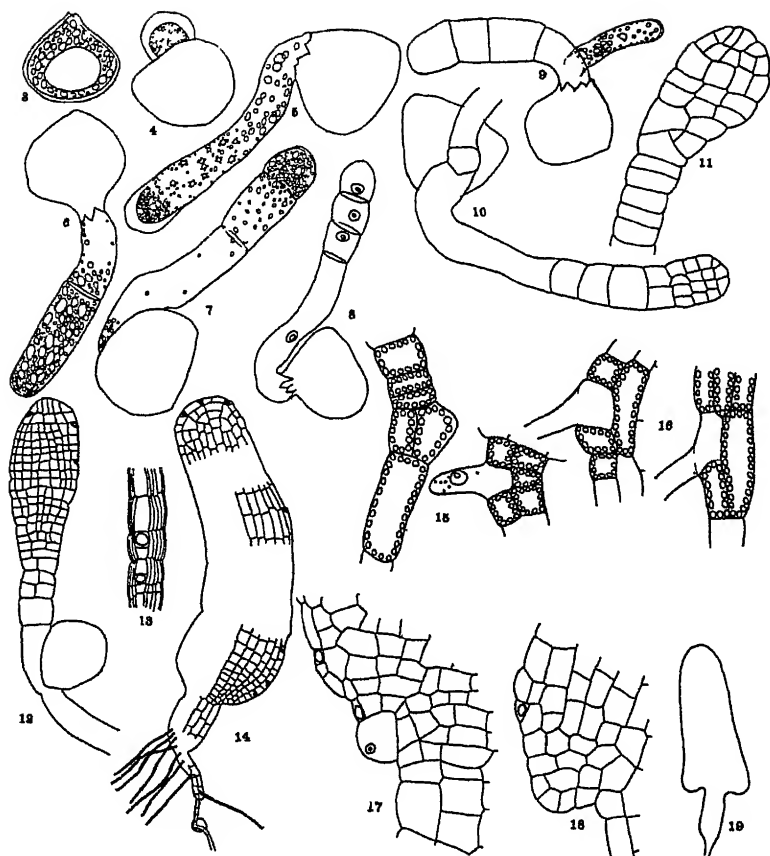
days. Heating the spores in water before the culture was made was found to hasten germination of air-dried spores recently collected, but often to be detrimental to older spores. Strong sunlight was found advantageous in the early stages of germination.

The spores of other species of *Riella* also require a rest period after apparent maturity. Germination resulted for HOWE and UNDERWOOD working with *R. americana* in the case of only a few of the spores collected several months previously. The same workers obtained more than 50 per cent germination of spores of *R. affinis* in a few days from specimens which had been in the herbarium for five and a half years. Spores of *R. paulsenii* germinated for PORSILD (11, 12) from mud collected about three years previously, and CAVERS (2) obtained germination of *R. capensis* from dried mud six years old.

In 1912, Dr. C. J. CHAMBERLAIN received from South Africa some dry soil from which a culture was made. Algae developed but no *Riella* made its appearance. *R. capensis* appeared very abundantly in a second culture, however, made from the same soil in 1925, 13 years after collection. Hundreds of plants were carried to the fruiting stage. This information was transmitted verbally by Dr. CHAMBERLAIN, with permission to quote it.

In several instances intracapsular germination was observed for the Texas species. Many of the spores germinated simultaneously, the germ tubes and rhizoids readily breaking through the slightly decomposed sporangium wall. Such germination was also seen by PORSILD (12) for *R. paulsenii*, and is mentioned by DOUTY (4) and GOEBEL (7).

There is little or no swelling of the spore of *R. americana* during germination; it remains essentially the same size and shape, and the depression at the middle of the inner face does not straighten or bulge out (fig. 2). Within two or three days after dried spores are placed under conditions favorable for germination, the wall becomes slightly more transparent and oil drops of various sizes are seen to occupy a large part of the interior of the spore (fig. 3). Just before rupture of the spore wall, a single large vacuole is formed opposite the middle of the inner face (fig. 3). The nucleus is not visible in unstained spores. The time required for the rupture varies from 4



FIGS. 3-19.—Fig. 3, outline of spore with wall about to rupture; fig. 4, germ tube emerging from under side of spore; fig. 5, sporeling before formation of first cross wall; fig. 6, sporeling after formation of first cross wall; fig. 7, after formation of second cross wall (all $\times 165$). Fig. 8, sporeling after formation of third cross wall, showing positions of nuclei; $\times 140$. Fig. 9, sporeling showing origin of primary rhizoid; distal cell slightly green, other two yellowish; fig. 10, division of distal cells to form early stage of primary thallus; all cells green; primary rhizoid three times length of germ tube; fig. 11, further divisions of distal cells of filament; $\times 165$. Fig. 12, later stage of primary thallus; $\times 115$. Fig. 13, portion of primary thallus seen from edge, with two large oil cells; $\times 165$. Fig. 14, mature primary thallus; $\times 75$. Fig. 15, two stages of origin of secondary rhizoid; fig. 16, two figures showing relation of secondary rhizoid to mother cell and surrounding cells; $\times 165$. Fig. 17, intercalary apical cell at base of expanded portion of primary thallus; fig. 18, early stage of lateral intercalary outgrowth; $\times 165$. Fig. 19, primary thallus showing lateral outgrowth on both sides; twin plants result in such cases; $\times 37$.

to 18 days, the shorter periods being more common. The rupture takes place at the middle of the outer or more spiny face (fig. 2), which is the apex of a rounded pyramid. This point of rupture, previously reported by HOWE and UNDERWOOD, is the exception for the spores of bryophytes and pteridophytes, which usually germinate at the middle of the inner face, that is, at the junction of the radial cracks or ridges. Possibly this condition in *R. americana* is associated with the shape of the spore, the apex of the pyramid on the outer face representing the weakest part of the spore wall. Or, possibly it is a hold-over from an ancestral condition in which the spores of a tetrad cling together after maturity, as do those of *Sphaerocarpus* (CAMPBELL 1). The rupture is effected by portions of the spore wall being lifted away from the apex and raised more or less at right angles to the spore. The resulting opening is characteristically circular (fig. 1); occasionally triangular, square, or irregularly angled openings are found, but always with the two dimensions roughly equal.

The first structure to break through is the germ tube (fig. 4), although in rare instances the primary rhizoid makes its appearance first. The germ tube at first grows out straight from the spore, but usually before it reaches a distance of 100 μ it bends to one side (fig. 5). This is no doubt a reaction to the stimulus of light or gravity, for occasionally growth is straight outward, and at other times the tube follows the spore wall half way around the spore before growing away from it. The end wall of the tube is at first somewhat thick (fig. 4), but later is no thicker than the side walls. The germ tube reaches a length of 80–150 μ before the first cross wall is formed (fig. 6); under conditions of weak illumination a length of 500 or even 700 μ has been observed. The diameter of the germ tube is nearly uniform throughout, ranging from 26 to 44 μ with an average of 33 μ . Generally, however, it is somewhat narrow at the spore and slightly enlarged at the tip.

The contents of the germ tube consist largely of closely packed oil drops (fig. 6). Small granules are also visible. As the germ tube grows, the oil drops become smaller and more scattered, remaining most numerous toward the tip of the tube. Chloroplasts and rhizoids are not seen at this stage.

HOWE and UNDERWOOD were the first to report on the early stages of germination of any species of *Riella*. In both *R. americana* and *R. affinis* they found the germ tube emerging before the rhizoid; in the former species it always developed from the outer face near its middle, and in the latter the same was true in practically all cases. The shape of the opening in the spore wall is not described nor shown in the drawings. PORSILD (12) found that the spore of *R. paulsenii* was cracked open along a straight median line more than half way around the spore. He found that a simple germ tube was formed, the length and width of which are dependent upon light, being short and broad in well lighted cultures on filter paper or kaolin. DOUIN (4) shows, without comment, a sporeling of *R. clausonis* in which the spore is split open along a straight median line. Other investigators give no details whatever on this stage, or even fail to mention it.

Filamentous stage

At the time of formation of the first cross wall, 45-115 μ from the spore, the oil drops are more numerous toward the tip of the filament, and no chloroplasts are visible. Growth of the filament continues at its tip, which remains rounded, and soon a second cross wall is formed (fig. 7) about midway between the first one and the tip. Growth continues in this manner until four to seven cells are formed in a linear series (fig. 8). These cells have an average length of 35 μ and an average diameter of 33 μ . Even in those cases in which the filament had become enormously elongated before being divided by cross walls, the new cells formed were of the sizes indicated.

As growth continues, the oil drops become smaller and gradually disappear; chloroplasts make their appearance, taking their place (fig. 9). The plastids are sometimes found in the 1-celled stage, but more frequently are not seen until two or three cross walls have been formed. The terminal cell is the first to become yellow, and then green, and the greening proceeds cell by cell toward the spore. In no case were plastids seen between the spore and the nearest cross wall, this region of the germ tube remaining colorless and containing scattered small oil drops and granules.

The primary rhizoid is generally formed after the second or third cross wall has been laid down (fig. 9). It makes its appearance on

the convex side of the germ tube, between the spore and the nearest cross wall, close to or directly at the spore. From a circular area a globular protrusion is formed, somewhat smaller in diameter than the germ tube, and the rhizoid thus formed protrudes farther as a tubelike outgrowth, which bends back over the spore and grows in a direction opposite to that of the germ tube. It is a cylindrical, smooth walled structure of uniform diameter ($17-28\ \mu$, average $20\ \mu$), with a rounded, thickened end wall. Its lumen is continuous with that of the base of the germ tube. Only in rare instances was a single cross wall observed, always some distance from the spore. The protoplasm remains granular and is at first densely crowded with oil drops, which become scattered as the rhizoid increases in length, but which remain more numerous near the tip. The nucleus is found a short distance back from the tip.

The primary rhizoid elongates rapidly, while the filament slackens its rate of growth so that the rhizoid is soon much longer than the germ tube. No secondary rhizoids have been observed at this stage. The growth of this stage is completed in two or three days.

HOFMEISTER (8) states that in *Riella reuteri* a short filament is formed upon germination, but he presents no description nor figures. TRABUT (16) says nothing about the early stages of development of *R. cossoniana*, but he shows two figures (without indicating cells) which he calls the protonema, but which, as pointed out by HOWE and UNDERWOOD, are more likely plants originating from gemmae. HOWE and UNDERWOOD state that in *R. americana* the germ tube is divided by transverse walls into a single row of several cells. More detail is given by these writers for *R. affinis*, in which the first wall was found to be somewhat curved, with its convexity turned toward the spore. The part above contained most of the starch grains, and in the course of time began to show chlorophyll. The length of the germ tube from the spore wall to the curved septum was found to vary from 0.02 to 0.7 mm. SOLMS (13) states merely that a filament of cells is produced by *R. parisii*. PORSILD (12) found that in *R. paulsenii* the germ tube becomes divided immediately by cross walls, and that the first rhizoid usually develops at the first cross wall.

Primary thallus stage

A few days after germination, when the filament has reached four to seven cells in length, its end broadens and becomes rounder. The exact order of wall formation was not observed, but in a short time the end cell has divided longitudinally and transversely into several small cells, all in the same plane, forming a rounded flattened structure one cell in thickness (fig. 10). Soon the second cell from the tip undergoes similar divisions, then the third, etc., in basipetal succession (fig. 11). The result is that the cells are smaller and younger at the tip, and progressively larger and older toward the spore. All oil drops disappear, and the peripheral regions of each cell become closely packed with chloroplasts.

For a time the derivatives of each of the original cells can be followed distinctly (fig. 12). The new thallus is at first broader at its tip, and later broader at or just below its middle. The thallus at this stage is usually less than 1 mm. in length at its maximum, but one thallus 1.5 mm. long was observed. Its width varies up to 0.5 mm. (usually less), and up to 30 cells at its widest part. Its shape varies from almost circular to egg-shaped, elongated oval, or much elongated. Division is in two planes, so that the thallus remains one cell in thickness, although exceptions have been found rarely in which the middle basal region is two cells in thickness. The thallus is somewhat lens-shaped in cross-section; the marginal cells are 13–18 μ thick, and the cells become progressively thicker toward the center of the thallus, where they have the thickness of the original cells of the filament, 35 μ (fig. 13).

No apical cell has been observed in the thallus at this stage. The cells at the tip remain smaller and more active at first, but soon the thallus becomes meristematic throughout. As the thallus approaches its maximum size at this stage, a zone of cells extending across the base of the broadened portion remains more meristematic, and the cells above this may be slightly elongated.

Sometimes all of the cells of the germ tube are involved in the broadening of the thallus; more commonly one, and less frequently two, three, or four nearest the spore remain undivided for a time (fig. 12). With maturity of the thallus this proximal group of cells divides further in two planes to form a zone one cell thick, one to

four cells broad (usually two), and one to eight cells long (usually four to six). The cell nearest the spore may remain undivided. These cells become much elongated, and are no doubt efficient in the conduction of materials absorbed by the rhizoids (fig. 14).

Oil cells begin to form on the expanded portion of the thallus when this region is six to eight cells broad (fig. 12). They are formed almost exclusively in the marginal row of cells, certain of which cut off a small cell from the end or outer corner. Such a small cell is prevailingly triangular, although frequently wedge-shaped or rectangular. Shortly after its formation, a large oil drop of irregular outline appears within it, and often almost fills it. Although surrounding cells may continue to divide, oil cells are not capable of further division.

The primary rhizoid continues to elongate until it is two to six times the length of the plant from spore to tip of thallus.

Secondary rhizoids take their origin from the elongated cells below the meristematic zone. The first ones appear nearest the spore, and the later ones progressively at more distal points; none has been seen to arise from the meristematic zone or from the regions beyond. In number they vary up to eight. Their method of formation is characteristic. The proximal portion of a lateral wall of a cell bulges out and forms an outgrowth which becomes a cylinder of uniform diameter (fig. 15). The chloroplasts are lost early, and both cell and rhizoid remain colorless (fig. 16). The secondary rhizoids have the same diameter as the primary rhizoid, and soon approach its length. They also have a thickened, rounded end wall, are smooth, of uniform diameter, and have no cross walls. As the chloroplasts are lost, scattered oil drops appear, the protoplasm becomes granular, especially at the tip, and the nucleus is found a short distance back from it (fig. 16).

When the primary thallus has reached its maximum size, therefore, the plant consists of the following parts: the spore; the primary rhizoid, which extends in one direction; the base of the original germ tube, which extends in the opposite direction; a long narrow band of elongated cells from some of which extend the secondary rhizoids; and a broadened unistratose region containing oil cells at its margin and remaining meristematic at its base (fig. 14). The

growth of the primary thallus stage up to this point was found to require two to three weeks. The empty spore wall remains attached until the primary thallus is mature. By the time it becomes broken off it appears to be partially decomposed, is swollen, and is more transparent than before germination. Strong sunlight is often detrimental to the thallus after the filamentous stage, rendering it stunted and broad. In weak light the thallus may be long and ribbon-shaped.

Among the abnormal shapes observed were those in which the distal portion of the thallus was twisted at a sharp angle to the proximal portion, the median line of the two portions being continuous and straight. This was probably due to a change of orientation with regard to light.

Many observations have been made by other workers on the primary thallus stage of various species of *Riella*. On the whole their interpretations agree, the main controversy being centered on the presence or absence of an apical cell. That an apical cell is present near the tip of the thallus was believed by HOFMEISTER (8), and LEITGEB (10) did not consider the growing region intercalary. HOWE and UNDERWOOD (9), GOEBEL (5), SOLMS (13, 14), and PORSILD (12) find no apical cell, but instead either consider the entire broadened portion of the thallus meristematic, or else recognize an intercalary meristematic region. DOUIN (4) figures the primary thallus stage without showing cells, and without description. The latter investigator, as well as the others, gives descriptions of the primary thallus of the several species which correspond rather closely to that of *R. americana*.

Lateral outgrowth and differentiation of stem and wing

When the cultures were three to five weeks old, growth became localized at one or both sides of the margin of the intercalary region of growth. Usually the cells on one side and at the middle mature, while those at the opposite margin remain meristematic. If only the middle cells mature and both margins remain meristematic, however, twin plants result (fig. 19).

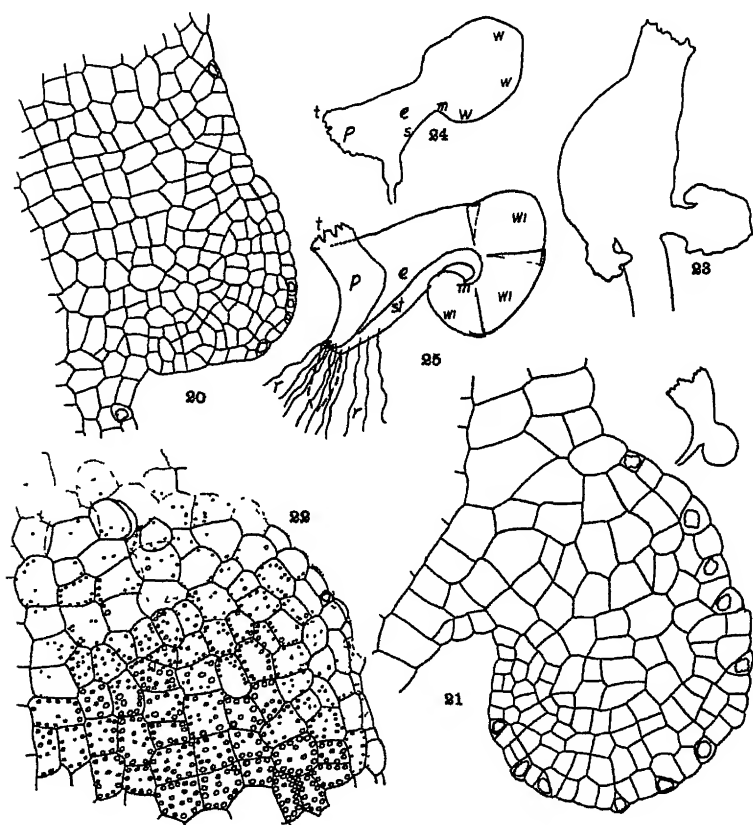
There is generally no indication of an apical cell along the meristematic margin, but sometimes a single large cell with small plastids, close to one or two oil cells, functions as an apical cell (fig. 17).

Even in these instances, however, the condition is purely temporary, the apical cell soon giving way to a number of small, actively dividing cells, which constitute a more permanent growing region (fig. 18). The cells of this region are the smallest ones of the thallus, except the oil cells. The smallest and most active of the meristematic group are those closest to the base of the thallus, that is, nearest the median line (proximal with reference to the spore). Walls come in irregularly at any angle, so that orientation of one cell bears no definite relation to that of any other, except that all cells lie in the same plane. The marginal cells, however, form a more definite row.

The new mass of tissue grows downward and outward so as to form an angle of 45° – 60° with the elongated cells below the meristematic zone, and it maintains throughout a position in the same plane as the primary thallus. A semicircular mass of tissue is soon formed (figs. 19, 20), which later becomes an almost circular outgrowth one cell in thickness (fig. 21). The cells of the adjacent primary thallus become somewhat distorted. As lateral growth continues, the primary thallus is bent to one side until it may occupy a position at right angles to its original axis, and the new growth also extends out more nearly at right angles to the original axis of the thallus (fig. 21). The cells at the base of the new growth elongate and carry the meristematic zone some distance from the primary thallus. At the same time an overgrowth occurs just beyond, on the proximal side (that is, toward the spore). This overgrowth extends around the tip of the meristematic region and back toward the primary thallus, nearly parallel with the axis of the new outgrowth, on its distal side (away from the spore). This results in the lateral outgrowth having a falcate structure which is similar, in miniature, to the shape of the adult plant, the most active cells being in the notch of the overgrown portion, and the cells between the notch and the primary thallus showing a tendency toward elongation (figs. 24, 25). Oil cells at first are not so numerous in the region of active growth, but appear on the margin of the falcate area some distance from the growing zone.

During progress of the lateral outgrowth, the primary thallus undergoes further changes. The cells at its tip lose their chloroplasts, protoplasmic granules become fewer, the cells separate at the cor-

ners and sides and come to overlap each other, their lumina finally become empty, and the walls break down and become detached



FIGS. 20-25.—Fig. 20, later stage of lateral outgrowth; $\times 145$. Fig. 21, circular stage of lateral outgrowth; primary thallus bent to one side and partially resorbed; large figure $\times 210$. Fig. 22, resorption at tip of primary thallus; $\times 145$. Fig. 23, abnormal secondary development of primary thalli from original primary thallus (one shows single lateral growing region, the other shows two); fig. 24, juvenile thallus shortly before differentiation of stem and wing; $\times 32$. Fig. 25, juvenile thallus after stem and wing have become differentiated (diagrammatic): *e*, region of elongated cells; *m*, region of most active division; *p*, primary thallus; *r*, secondary rhizoids; *s*, region destined to become stem; *st*, stem; *t*, resorbed tip of primary thallus; *w*, region destined to become wing; *wi*, wing; $\times 13$.

from the remainder of the thallus (fig. 22). This breakdown of the cells continues progressively from the tip toward the base of the

primary thallus. It usually begins at the same time as the formation of the lateral outgrowth, but it has been observed earlier. It continues until, by the time the stem and wing are differentiated, two-thirds or three-fourths of the primary thallus is broken down. The process is interpreted as being a phenomenon of resorption with the passage of the resorbed materials to the new growing region.

In place of the lateral outgrowth just discussed, a second thallus of the same type as the primary one may occasionally develop from the lateral meristematic region; such a thallus in its turn develops a slender stalklike portion and a broadened group of cells, at the base of which one or two lateral meristematic zones develop, just as on the primary thallus (fig. 23).

Under the cultural conditions discussed, the lateral outgrowth developed slowly, many of the plants died, and many others were destroyed during examination. The cultures also became overcrowded with algae, which were detrimental to the growth of *Riella*. It was further found that the narrow portion at the base of the primary thallus was easily broken off. It has been possible, to date, to grow only a single specimen to the stage of showing clearly the differentiation of stem and wing; this specimen was found 80 days after the cultures were sown (fig. 25).

The stem or axis appears on the proximal edge of the lateral outgrowth, that is, on the edge nearer the spore, and extends from the meristematic zone at the notch of the lateral outgrowth to the base of the primary thallus. This is accomplished by division of these marginal cells, which are already elongated, in the third plane to form an axis which is circular or oval in cross-section. This is the first time in the life history of *Riella* that cell divisions normally take place in the third plane. The falcate structure already described as extending from the growing point over the crest and back to the primary thallus constitutes the wing. The stem, therefore, is on the proximal margin of the lateral outgrowth (toward the spore), and the wing on the distal side (away from the spore). There is no sharp line of demarcation between the primary thallus and the new outgrowth, both of which lie in the same plane. The boundary line can be determined roughly by the fact that the cells at the base of

the new growth are somewhat larger than those of the adjacent primary thallus.

The plant at this stage is 2.6 mm. long from the base of the lateral outgrowth to the crest of the wing, and the maximum width of the wing is 0.44 mm. Since the margin of the wing has elongated more rapidly than the stem, it is a much folded and wavy structure, but shows no evidence of spiral twisting around the stem, as was at first indicated for *R. helicophylla*. The stem is 0.07 mm. wide at its distal curved region near the meristematic zone, and 0.18 mm. along most of its course, which is straight; along this straight portion it is five to seven cells wide. A portion of the primary thallus is still attached, and new rhizoids have developed from the base of the stem. There is no indication of sex organs, but trichomic outgrowths have made their appearance in the region of the growing point.

The early workers with *Riella* were much confused by the lateral outgrowth from the primary thallus and by the differentiation of the stem and wing, and their interpretations have differed materially. HOFMEISTER (8) calls attention to a lateral growing point, but he apparently thought of a growing region nearer the apex of the primary thallus; no apical cell is mentioned. He did not succeed in growing the sporelings to the point of stem and wing differentiation. TRABUT (15) does not discuss this stage, but his figures show what seems to be the primary thallus with a lateral outgrowth. GOEBEL (5) had no sporelings available. HOWE and UNDERWOOD (9) did not succeed in growing sporelings beyond the early primary thallus stage; they thought, apparently incorrectly, that the upper portion of the primary thallus becomes the wing and the lower portion the stem. They thought that the stem in *R. affinis* was being formed as a result of divisions in the third plane at the base of the primary thallus; their illustrations, however, show small, lateral, intercalary outgrowths comparable with those found by the writer for *R. americana*, although no interpretation was made of these. SOLMS (13) found two lateral outgrowths at the base of the primary thallus; one of these continues to grow while the other is suppressed. He found the growing region in the new growth, but thought that its entire lower portion becomes the stem. He maintained that an apical cell is formed later, but that, in spite of its position, it cannot be con-

sidered intercalary. His plants, however, did not actually reach the stem stage. PORSILD (12) found the lateral outgrowth from the primary thallus, and its thickening to form the stem on the side nearest the spore, but he found no apical cell at any stage of development, not even after the stem and wing had been clearly differentiated. He also reported the presence of oil cells on the primary thallus, but found less regularity in their shape than is reported in the present paper. He, too, found that the cells of the primary thallus die and empty their contents after differentiation of stem and wing. A sharp boundary line is reported to exist between the primary thallus and the wing. GOEBEL (6) found one or two growing regions localized at lateral intercalary points. He found no sharp line of demarcation between the primary thallus and the wing, hence he does not consider the lateral outgrowth (stem and wing) as a new structure, different from the primary thallus, as was thought by SOLMS and PORSILD. He found no definite wedge-shaped apical cell in the meristematic region at the tip of the stem.

Symmetry

The peculiar position of the wing with respect to the stem has been the subject of much discussion. A part of the dispute over the question of symmetry has been based on conclusions from meager information. It has been pointed out that many of the workers had no sporelings at their disposal, or else did not succeed in growing them to a sufficiently adult stage to show the origin of the stem and wing. Furthermore, the terms symmetry, dorsal and ventral, and distal and proximal, have not always been clearly defined.

In the filamentous stage, the *Riella* sporeling is evidently radially symmetrical, all sides of the short filament having the same appearance. The factor which determines the angle of the first longitudinal wall in the filament has not been determined; probably it will be found to be light or gravity.

The primary thallus stage of *R. americana* is certainly bilaterally symmetrical; that is, a plane extending through the middle of the thallus, perpendicular to it, and from its distal point to its proximal region, divides it into halves which image each other. This bilateral symmetry remains until the lateral outgrowth begins to develop on

one side; or, in the case of development of lateral outgrowths simultaneously on both sides of the primary thallus, bilateral symmetry is evident for a much longer time. There is no reason for considering the flattened, unistratose primary thallus dorsiventral, if by that term is meant that there is a difference between the two faces, for the exposed faces of the cells are alike on both sides of the thallus.

Other species of *Riella* exhibit the same condition in the primary thallus stage, as indicated by the descriptions and figures of HOFMEISTER (8), GOEBEL (5, 6), HOWE and UNDERWOOD (9), SOLMS (13), and PORSILD (12). A difference may exist in those species which grow horizontally on the substrate. Furthermore, GOEBEL (6, 7) drew a close parallel between the primary thallus of *Riella* and the gemma of *Marchantia* or *Lunularia*: in both of these cases there are one or two lateral intercalary growing regions from which the adult thalli arise; both gemma and primary thallus grow vertically at first; and the two flattened surfaces of both gemma and primary thallus are alike, the two faces of the gemma becoming different only after this structure has been in contact with the substrate for a time.

Confusion in the terminology of symmetry is mostly in evidence in connection with the lateral outgrowth, the young plant with differentiated stem and wing, and the adult plant. HOFMEISTER (8) compared the adult plant with the thallus of *Marchantia* in which one of the wings has been suppressed. LEITGEB (10) considers the wing a dorsal outgrowth of the stem, comparable with a comb standing at the tip of the stem; such a conception, however, implies that the stem develops before the wing, which is not the case. GOEBEL (5) recognizes some similarities to the thallus of *Marchantia*, but places emphasis on the fact that *Riella* grows in an erect position from the beginning (his "Profilstellung"). In a later work (7) he states that the apparently radial thallus is in reality an extraordinary modification of the usual dorsiventral thallus of the liverworts. Those species with a creeping, horizontal stem differ from the usual form of the liverworts only in that the wing is developed in a vertical position. The relationship between the wing and stem is similar in the species which grow in an erect position. He therefore considers the wing as dorsal to the stem. HOWE and UNDERWOOD consider the adult plant bilaterally symmetrical in the plane of the wing,

which is dorsal in relation to the stem. CAVERS (3) considers the wing dorsal and the stem ventral.

In the discussion of the lateral outgrowth and the adult plant of *R. americana* which follows, an effort is made to clarify the terminology of symmetry. The lateral outgrowth of the American species is from the beginning irregular, the cells nearest the spore (proximal) being smaller than those farther away (distal). The shape of the outgrowth is not perfectly semicircular, nor later absolutely circular; no plane at right angles to its surface will divide it into parts which image each other. The same is true for the older plant, in which stem and wing have been differentiated, and also for the mature plant. If the lateral outgrowth or the adult plant can be considered bilaterally symmetrical at all, it must be in the plane of the wing, as noted by HOWE and UNDERWOOD. Dorsiventrality, as just defined, is not exhibited at any stage in the development from the beginning of the lateral outgrowth to the maturity of the plant, for the two faces of the young lateral outgrowth, as well as of the stem and wing of the older plants, are alike.

As the lateral outgrowth develops, the angle which it forms with the primary thallus varies greatly; but as its development continues, a simple bending by differential growth on two opposite sides of the stem results in the adult plant being erect or ascending. In nature the mature *R. americana* is a much branched plant; only a few of the branches are definitely vertical, while most of them are ascending at various angles from 45° to 90° with the substrate. It is therefore logical to discard the terms dorsal and ventral, as used by most workers with *Riella*, who consider the wing dorsal and the stem ventral. A better terminology is to consider the wing distal with regard to the stem, using the position of the spore as a point of reference.

Summary

1. Spores of *Riella americana* were germinated in water and studied mainly in the living condition. There is little or no swelling of the spore, but oil drops are present in great numbers and a large vacuole forms at a position opposite the point of rupture.

2. The spore ruptures at the middle of the outer face, and a germ tube containing great numbers of oil drops emerges through the

spore wall, leaving a rounded opening. By apical growth a filamentous stage is produced, consisting of four to seven cells, which soon show numerous chloroplasts. The primary rhizoid originates from the germ tube between the spore and the nearest cross wall.

3. Beginning with the apical cell, most of the cells of the filament divide by longitudinal and cross divisions to form a broadened primary thallus one cell in thickness, the base of which remains as a meristematic zone. The secondary rhizoids develop as extensions of some of the cells between the spore and the broadened primary thallus. All rhizoids are smooth, colorless, and of uniform diameter.

4. The meristematic zone becomes localized at one or both sides, and one or two lateral outgrowths result which are one cell thick and in the same plane as the primary thallus, the tip of which is resorbed. The distal portion of the lateral outgrowth (away from the spore) becomes the wing of the adult plant, and the proximal margin (toward the spore) thickens by cell divisions in the third plane to form the stem. By a bending of the axis of growth the adult plant becomes erect or ascending.

5. The filamentous stage is radially symmetrical, and the primary thallus stage bilaterally symmetrical. The later stages, from the beginning of the lateral outgrowth to the adult plant, are not dorsiventral and are bilaterally symmetrical only in the plane of the wing. The wing is simply considered distal to the stem, using the position of the spore as a point of reference.

The writer wishes gratefully to acknowledge the help of Dr. W. J. G. LAND during the progress of the work and in the preparation of the manuscript.

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[Accepted for publication December 16, 1930]

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REGENERATIVE CAPACITIES OF LEAF AND LEAF- LET CUTTINGS OF TOMATO AND OF LEAF AND SHOOT CUTTINGS OF POTATO

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(WITH TWENTY-THREE FIGURES)

Introduction

It is well known that the tomato plant is easily regenerated by stem cuttings, but available literature fails to reveal any work on the regenerative power of leaf and leaflet cuttings. Such an investigation was made by the writer during 1928 and 1929. Both leaf and leaflet cuttings readily regenerated roots, but only the former regenerated shoots.

The responses of leaf and leaflet cuttings of the tomato suggested the possibility that leaf cuttings of the Irish potato possessed similar powers of regeneration. KUPFER¹ reported that leaf cuttings of the Irish potato gave three distinct types of response: regeneration of roots; regeneration of a true tuber; and enlargement of the end of the petiole into a starch-bearing, tuber-like organ that might or might not regenerate roots. The results of experiments conducted in 1929 indicated that entire leaf cuttings, including no part of the stem, do not regenerate true tubers. This did not entirely agree with KUPFER's interpretation of results with leaf cuttings. To check the results obtained in 1929 as against those of KUPFER, the experiments were continued in 1930. This paper reports the results of investigations with the tomato during 1928 and 1929, and with the Irish potato during 1929 and 1930.

Materials and methods

All material was shaded, protected from drying winds, and handled as rapidly as possible from the time it was collected until the cuttings were placed in the propagating bench. Sand was packed around the bases of the cuttings as is usually done with those of

¹ KUPFER, ELSIE. Studies in plant regeneration. Mem. Torr. Bot. Club 12:195-241. 1907.

softwood. Specimens were removed from the propagating bench at frequent intervals and examined, care being taken to protect them from injury from drying. The bench was exposed to the sun except very late in the afternoon. A glass sash cover, used to maintain high humidity during the daylight hours, especially during the hottest part of the day, was removed at night.

Investigations

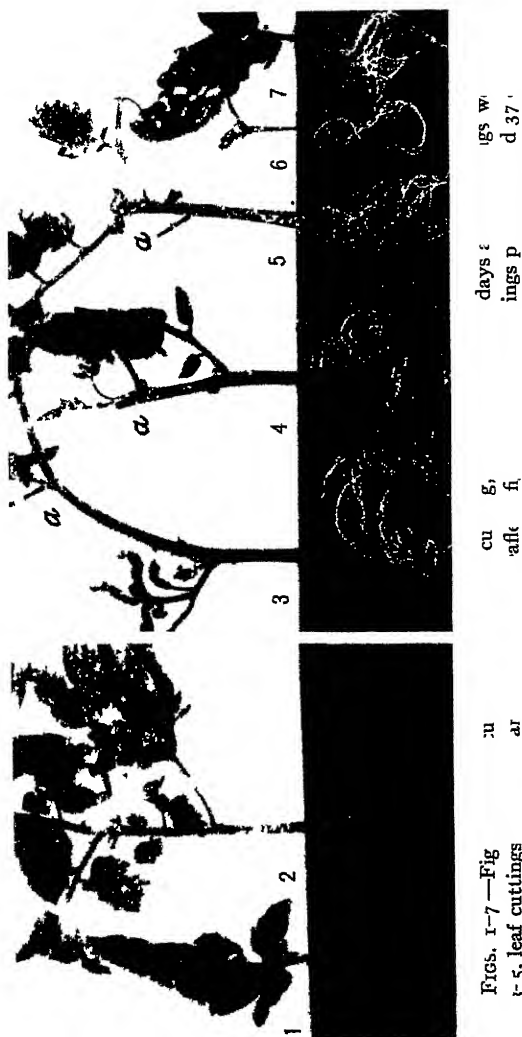
I. *LYCOPERSICUM ESCULENTUM*

Cuttings consisting of entire leaves, carefully removed from the plant so that no part of the stem was included, were made in August, 1928. In May, 1929, additional cuttings of this type were made as well as others consisting of leaflets. After roots were produced, the plants were either potted and grown in the greenhouse or planted and grown in the garden.

Roots were readily regenerated by both leaf and leaflet cuttings, while only leaf cuttings regenerated shoots. Ten to fifteen days were required for the cuttings to become well rooted. Most of the roots appeared from the callus at the base of the petiolules and petioles, as shown in figs. 1 and 2 respectively. A few appeared on the petioles and petiolules above the callus, as shown in figs. 5 and 7 respectively.

Shoots began to appear on the leaf cuttings in the axils of the leaflets soon after the cuttings were potted or planted. The new shoots tended to develop toward the terminal parts of the leaf cuttings (fig. 3*a*), unless the cuttings were headed back, in which case they appeared in the more terminal axils remaining (figs. 4, 5). Each shoot developed a decidedly club-shaped enlargement toward its base, but there was a necklike constriction at the point of its attachment to the rachis. As a result of this constriction, the union of the shoot and rachis was usually not very strong (fig. 4). As the new shoot developed, the petiole and rachis enlarged and served as the main stem for the new plant (fig. 8*a*). The enlargement occurred rather slowly, as is typical of the basal part of the stems of stunted or hardened tomato plants. Some of the cuttings which had differentiated shoots in the axils of the leaflets later differentiated one or more shoots from the stem near the surface of the soil. Frequently these shoots became the most vigorous and formed the main part of

the plant. The leaflets of the leaf cuttings functioned as foliage until the developing plant unfolded new leaves, and for a considerable



FIGS. 1-7.—Fig
1-5, leaf cuttings
6-7, plants made.

time thereafter; but as the new plant increased in size and age the original leaflets gradually withered and died (fig. 8*b*), as do the more basal leaves of a normal tomato plant.

While leaflet cuttings formed roots as did leaf cuttings, they did not differentiate shoots (figs 6, 7). Although kept under observation a month or more longer than the time required for leaf cuttings to differentiate shoots, it is possible that the leaflet cuttings were not observed long enough to determine definitely whether they



FIG 8.—Plant from leaf cutting *a*, petiole and rachis developed into main stem, *b*, original leaflets

would differentiate shoots; the environmental conditions, moreover, may not have been favorable for shoot differentiation

No special effort was made to obtain fruits from any of the plants regenerated by the leaf cuttings. Some of the cuttings taken in 1928, however, were kept in the greenhouse while others were planted in the garden. Flowers and fruits were produced by plants grown in both locations (fig. 8).

II. SOLANUM TUBEROSUM

EXPERIMENT I —Early in the summer of 1929, an experiment was started with leaf cuttings of the Triumph variety. Within 3 weeks, despite injury to the foliage by a fungus, a number of the cuttings had formed roots and one had formed a tuber near the base of the petiole. The latter response suggested that since the tuber is an enlarged stem, there might have been left on the petiole of the cutting a rudimentary bud which developed into the tuber.

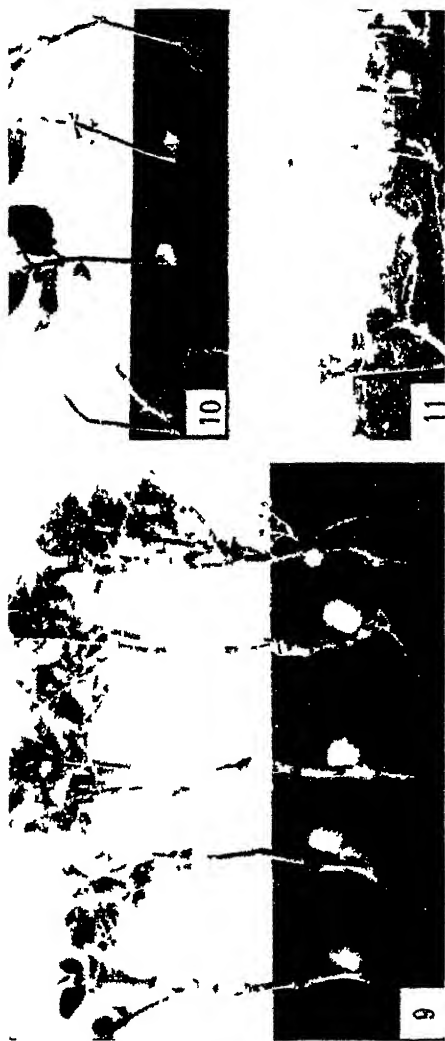
EXPERIMENT II —To determine whether the tuber produced by one of the cuttings in experiment I was the enlargement of an axillary bud, three types of cuttings were taken July 1, 1929, from a late planting of the Irish Cobbler variety. These cuttings consisted of (1) the terminal 4-6 inches of stems with attached buds and leaves; (2) entire leaves with axillary buds carefully removed; and (3) entire leaves with a heel of the stem and axillary buds. These cuttings were handled as in experiment I and were similarly injured by a fungus. They were kept in the propagating bench until July 27, when they were examined and some of them photographed.

None of the cuttings without axillary buds developed tubers or shoots, whereas all of the others developed either a shoot, tuber, roots, or more than one of these in combination (figs. 9-11). The roots usually occurred on the new shoot, but a few stem and leaf cuttings with a heel of the stem regenerated roots at the base. The fact that cuttings with axillary buds readily regenerated tubers, while cuttings without axillary buds did not, strongly indicated that the tuber produced by one of the cuttings in experiment I developed from an axillary bud which had been overlooked and left on the base of the leaf petiole.

EXPERIMENT III.—To check the results obtained in experiments I and II, and the observations reported by KUPFER, another experiment was started April 14, 1930. Forty entire leaf cuttings with axillary buds, and 35 without axillary buds, were made from the Triumph variety. Leaves of different ages and from different locations on the plant were used in each case.

Ten days after the experiment was started 37 of the 40 cuttings with axillary buds had converted the axillary bud into one or more tubers (fig. 12). One had died from petiolar infection; the other two,

representing small young leaves, had not produced tubers. None of the cuttings without buds had produced tubers, but the basal part



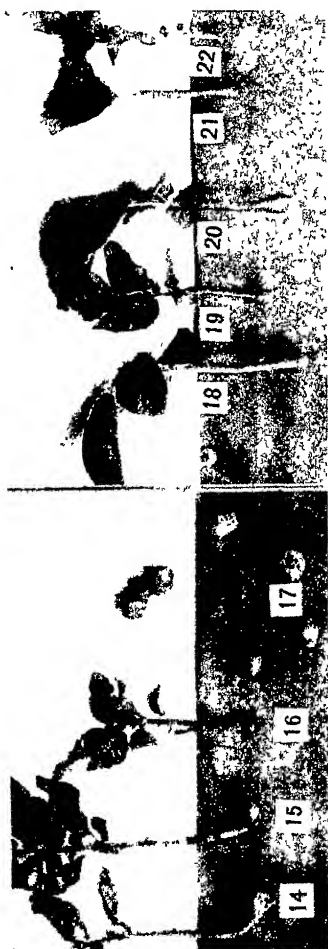
FIGS. 9, 10, 11.—Fig. 9, stem cuttings with axillary buds; fig. 10, leaf cuttings with axillary buds; fig. 11, leaf cuttings with healed basal part.

of the petiole of some of them was enlarging (fig. 13) and developing the pink color characteristic of the skin of the Triumph potato. Thirty-two days after the experiment was started, all with axillary

buds, except the one that died early, had developed one or more tubers (figs 14-17). The foliage of most of the cuttings having died,



FIGS. 12, 13.—Fig. 12, leaf cuttings with axillary buds, photographed 10 days after cuttings were made; fig. 13, leaf cuttings without axillary buds, photographed 10 days after cuttings were made



FIGS. 14-22.—Figs 14, 15, potatoes developing from axillary buds and foliage dying, fig. 16, very young when cutting was taken; fig. 17, potatoes developed from older and larger leaves that died, photographed when 32 days old. Figs. 19, 20, 21, leaf cuttings without axillary buds; fig. 22, leaf cutting with axillary bud; side views, photographed when 32 days old.

the potatoes were harvested and weighed. They ranged from 0.105 to 5.805 gm., with an average weight of 2.61 gm. per plant. Some of the potatoes were allowed to cure and to go through a rest period

for 34 days, during which time they were exposed to light and developed chlorophyll. These were then planted in potting soil. Within 13 days after planting, some of the eyes were beginning to swell, and in 30 days the terminal bud had started growth. A photograph was made of one of these on August 3 (fig. 23). One of the potatoes produced from an axillary bud was overlooked and left in the propagating bench. It produced a shoot July 17, 1930.

The foliage of practically all of the cuttings without axillary buds was alive 32 days after the experiment was started. None of these cuttings had developed tubers, but the basal part of the petiole of several had enlarged considerably, the enlargement occurring (except in one instance) only opposite the axillary side (figs. 18-21). The cuttings were kept in the propagating bench and examined from time to time for 66 days, when all tops were dead. The enlarged bases were taken up and an attempt was made to let them go through a rest period preparatory to making a sprouting test, but all shriveled and died soon after they were removed from the soil.

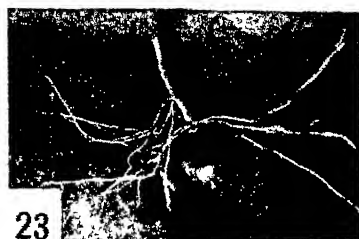


FIG. 23.—Tuber produced from leaf cutting giving rise to normal plant.

Discussion

The axillary areas located at the bases of the more terminal leaflets of tomato leaf cuttings possess the power of terminal dominancy, and express it by regenerating shoots more quickly than similar but more basal axillary areas. The axillary areas of the most terminal leaflets remaining on headed-back leaf cuttings exhibit terminal dominancy by regenerating shoots more quickly than the more basal

axillary areas. These responses are interesting because of their similarity to the dominancy expressed in many plants by terminal buds and buds just below pruning cuts.

The behavior of the tomato leaf cuttings differed from the potato leaf cuttings in that the former readily regenerated roots, and also regenerated shoots in a comparatively short time, while true leaf cuttings of the latter regenerated few or no roots and failed to regenerate shoots. The regenerative responses of leaf cuttings of the potato not only differed greatly from the responses obtained with leaf cuttings of the tomato, but the former gave different responses, depending on whether the cutting carried an axillary bud.

There was a general tendency for the larger, more basal leaf cuttings to make larger tubers and to make them more quickly (figs. 12, 14-17).

The rapidity with which leaf cuttings with axillary buds converted the buds into tubers and the size of the tubers produced in a given time are relative measures of the importance of the leaf area to the potato plant. These also suggest that potato leaves with axillary buds might be used in a laboratory method to determine the extent to which injury to the foliage, caused by certain insects or diseases or other causes, affects the metabolic processes concerned in shoot, root, and tuber formation. It appears, moreover, that this information might be used in a practical way to show the grower how essential it is to protect foliage from injury of any kind, especially with quick maturing plants like the potato. The foliage also died in a shorter period on the older and larger leaf cuttings. This difference in behavior supports the belief held by some that the amount of leaf area and the age of the cutting influence the amount of food manufactured and the nature of the regenerations obtained.

The basal part of the older leaf cuttings without axillary buds appeared to enlarge earlier and die more quickly than was true with the younger leaf cuttings. This is shown by different cuttings in figs. 18-21. Fig. 21 is the oldest leaf; its foliage has practically died and dried. The photograph of the material shown in figs. 18-22 was taken 32 days after the cuttings were made.

Some of KUPFER'S drawings are similar to figs. 18-20, and show the enlargement of the base of the petiole to be opposite the axillary area. Her drawing of a leaf cutting that produced a tuber showed

the tuber on the axillary side of the petiole. This is identical with the behavior of leaf cuttings with axillary buds shown in figs. 12, 14-17, 22. On these regenerated tubers small leaves and eyes may be noted; KUPFER's illustration also shows rudimentary leaves and possibly eyes. The petioles which enlarged at the base and formed a potato-like skin failed to develop leaves or eyes, while the tubers from cuttings with axillary buds formed both. Potatoes produced from axillary buds went through a rest period, and had the power to develop green color in the presence of light and to grow when planted; whereas enlarged petioles without axillary buds died when taken up. Therefore, results of experiments reported in this paper strongly suggest that KUPFER misinterpreted some of her data on regeneration with Irish potato leaves. The tuber produced by a leaf cutting in her studies was presumably developed from an axillary bud which was unknowingly left on the cutting. It may be possible to produce tubers under some conditions, and with some varieties of leaf cuttings, without axillary buds, but observations made during the experiments herein reported are to the effect that, should this occur, the tubers would most likely develop on the petiole opposite the axillary side.

Summary

1. An investigation was made to determine the regenerative capacities of leaf and leaflet cuttings of the tomato, and of stem and leaf cuttings of the Irish potato.

2. Leaf cuttings of the tomato regenerated roots from the petioles, and shoots in the axils of the leaflets and from the petiole. Some specimens produced flowers and fruits. Leaflet cuttings regenerated roots but had not differentiated shoots when the experiment was discontinued.

3. Stem cuttings of the potato converted one of the axillary buds into a tuber, tuber and roots, or a tuber in combination with a shoot and roots. Some produced roots directly from the base of the cutting. Potato leaf cuttings with axillary buds converted the axillary bud into either a shoot or a tuber.

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[Accepted for publication January 22, 1931]

NEW OR OTHERWISE NOTEWORTHY
COMPOSITAE. VII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 421

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BIDENS KILIMANDSCHARICA retrorsa var. nov.—A specie achae-niorum aristis ad apicem saepissime retrorsum hamosis differt.

Specimens examined: A. E. Haarer 1472, at altitude of about 4000 feet, Doloti, Moshi District, German East Africa, August, 1928 (type in Herb. Kew).

BIDENS DIVERSA megaglossa var. nov.—A specie bracteis exterioribus ciliatis, ligulis 7–13 mm. longis differt.

Specimens examined: Newton, Biballa, Serra da Chella, Angola, Portuguese West Africa, June 3, 1883 (2 type sheets in Herb. Berl.).

Bidens mossii nom. nov.; *Coreopsis tripartita* M. B. Moss, Kew Bull. 1929:184 and 196. 1929.—A species deceptively like *Bidens whytei* Sherff, from which it appears to differ, however, in its larger and less dilated exterior involucre bracts, its exaristate achenes, etc.

BIDENS SETIGERA var. *abyssinica* (Schz. Bip.) comb. nov.; *Chrysanthellum* (subgenus *Microlecanium*) *abyssinicum* Schz. Bip., Flora 25:440. 1842; Walpers Repert. 6:171. 1846; *Microlecanium abyssinica* (Schz. Bip.) Benth. & Hook. ex O. Hoffm. in Engler und Prantl Pflanzenfam. 4^v:240, fig. 118C. 1894.

The achenes of this variety lack aristae but have the pappus more or less definitely coroniform or cup-shaped (whence the name *Microlecanium* given by SCHULTZ BIPONTINUS). TEGETMEYER (O. Hoffm. loc. cit.) has illustrated this peculiar pappus in its extreme development.

In his own original treatment SCHULTZ BIPONTINUS referred this form, as representing a subgenus, to *Chrysanthellum*. The affinity with that genus seems, however, purely fallacious. BENTHAM and HOOKER (loc. cit.) elevated the subgenus, which SCHULTZ BIPONTINUS had named *Microlecanium*, to the rank of a genus separate from *Chrysanthellum*. However, through *Bidens setigera* Sherff and *B.*

setigeroides Sherff the type material of *Microlecania* is too closely allied with *Bidens* to justify generic segregation. Thus, for example, *B. setigera* has very long, slender aristae and many of the achenes are much the same as in other species of *Bidens*; but a fair percentage of them have the shorter or secondary pappus noticeably modified into an annular or cupuliform corona, thus suggesting the so-called *Microlecania* material, here treated as the var. *abyssinica*. All things considered, it seems that the var. *abyssinica* bears somewhat (although not entirely) the same relationship to *B. setigera* proper that *Bidens aristosa* var. *mutica* (Gray) Gattling. bears to the species *B. aristosa* (Michx.) Britt. proper.

BIDENS TRIPLINERVIA var. *MACRANTHA* octoradiata f. nov.—E var. *macrantha* capitulis regulariter 8-radiatis differt.

Specimens examined: *R. Hauthal* 273, alt. 3600–4800 m., vicinity of La Paz, Bolivia, January, 1906 (Herb. Berl.); *Pflanz* 406, alt. 3550 m., schist slope, Chullo, Palca, La Paz, Bolivia, Mar. 13, 1910 (type in Herb. Berl.); *A. Mathews* 571, Peru, April (Gray); *Eduard Seler*, Sierra Chica, Province of Córdoba, Argentina, Apr. 1, 1910 (Herb. Gray); *idem et Mrs. Caecilie Seler* 3021, alt. 3000 m., Cordillera between Todos los Santos and Chiantla, Guatemala, Sept. 11, 1896 (Herb. Berl.); *A. Stübel* 43b, Peru, April–June, 1875 (Herb. Berl.), *Dr. A. Weberbauer* 435, alt. 3700 m., Peru, Feb. 24–25, 1902 (Herb. Berl.).

Bidens triplinervia H.B.K. and its varieties are ordinarily separable from *B. andicola* H.B.K. and its varieties by means of the number of rays to each head. In the former these number usually five, in the latter usually eight. In the case of *B. triplinervia* var. *macrantha*, however, there occur very rarely forms in which all the heads are eight-rayed.

BIDENS GRACILIOR ukerewensis var. nov.—Folia bi-tripinnatifida, segmentis ultimis linearibus moderate adpresso-hispidis plerumque 1–1.5 mm. latis; achaeniis exterioribus corpore circ. 4.5–5 mm. longis interioribus 6–7 mm. longis, omnibus tenuiter biaristatis aristis 0.5–1 mm. longis apice retrorsum 1–4-hamosis.

Specimens examined: *Dr. Carl Uhlig* 19, abundant along the shoreline of Lake Victoria Nyanza (L. Ukerewe), Ukerewe, German East Africa, Apr. 20, 1904 (type in Herb. Berl.).

BIDENS SUBALTERNANS DC. Prodr. 5:600. 1836; *B. platensis* Mang., An. Mus. Nac. Buenos Aires 24:230. 1913. —*Bidens platen-*

sis Mang. was described as a hybrid between *B. pilosa* L. (staminate) and "*B. bipinnata* L." (pistillate). A study of MISS MANGANARO'S text some time ago convinced me that the plant which had been assumed to be *B. bipinnata* L. was in reality *B. subalternans* var. *simulans* Sherff. Recently I was given valuable aid in the determination of *B. platensis* itself by PROFESSOR ANGEL L. CABRERA of the La Plata Museum in Argentina. In a letter he wrote to the following effect: His numerous searches in the past for the type of *B. platensis* had been fruitless. He therefore had given the type up for lost. Recently, however, he had been entrusted with the rearrangement of the SPEGAZZINI collections for the La Plata Museum. Now, the late MISS MANGANARO had been a pupil of SPEGAZZINI, and at her death her parents sent her collections to SPEGAZZINI. PROFESSOR CABRERA found her collections inside an old chest, in November, 1930. The herbarium was in great disorder, without labels or any other indications. However, there were several specimens of *Bidens* that had been very carefully mounted, and these were "closely similar to the original figure of *B. platensis*." Since no other specimens in the Manganaro collection had been given such special care, PROFESSOR CABRERA concluded (doubtless rightly) that these specially mounted ones represented the type material of *B. platensis*. He generously lent me two of the supposedly type specimens for study and they are now before me. Both are typical *B. subalternans* DC.

Bidens phelloptera sp. nov.—Herba verisimiliter perennis, usque ad 2 m. alta. caule obscure angulato glabro. Folia secundaria (primaria non visa) tenuiter petiolata petiolis ∓ 1.5 cm. longis petiolo adjecto ∓ 4.5 cm. longa, pinnatim 3–5-partita, segmentis valde membranaceis ovatis vel lanceolatis, apice acutis, supra glabratis subtus pubescentibus, circ. 6–13 mm. latis, acerrime dentatis dentibus mucronatis. Capitula corymbose disposita, ramulos plerumque glabros usque ad 8.5 cm. longos terminantia, radiata, pansa ad anthesin ∓ 4 cm. lata et circ. 10–11 mm. alta. Involucri glabri bracteae exteriores 8–10, lanceolatae vel lineari-oblongae, apice obtusae, mox valde reflexae; interiores late oblongo-lanceolatae, basaliter vel etiam usque ad medium connatae, demum circ. 8.5–9.5 mm. longae. Paleae late lineari-oblongae, apicaliter obtusae atque atro-coloratae, 7–9.5

mm. longae. Flores ligulati ∓ 6 (normaliter 8?), flavi, ligula anguste obovati, apice minute acriterque 3-4-denticulati, 1.7-2 cm. longi. Achaenia valde obcompressa, unaquaque duarum facierum subatra 8-sulcata glabra vel apicem versus erecte setosa, marginibus crassiusculis densissime erecto-ciliata, exteriora late oblonga 5-6 mm. longa et marginibus perspicuis incrassatis inclusis 2-2.4 mm. lata apicaliter calva vel breviter biaristata et saepe paucisetosa, interiora lineari-oblonga corpore circ. 8-9 mm. longa et marginibus angustis inclusis circ. 1.5-1.8 mm. lata, apice plus minusve setoso biaristata aristis tenuibus usque ad 2.5 mm. longis, nunc glabris nunc antrorsum vel etiam retrorsum 1-2-hamosis.

Specimens examined: Dr. W. Busse 2257, alt. about 750 m., south slope of Mt. Gonja, Usambara, German East Africa, Apr. 18, 1903 (type in Herb. Berl.).

The branches of the type material have internodes 1-1.5 dm. long. Busse's label lists the plant as an herb and gives its height as being up to 2 meters. The interior involucre bracts are connate below, often even up to the middle, thus simulating those of the allied genus *Thelesperma*. The general habit, however, in no way suggests *Thelesperma*. The achenes are thick-margined and suggest those of some species of *Corcopsis*, but the occasional retrorse awn barbs are foreign to that genus.

Bidens personans Degener & Sherff sp. nov.—Perennis, supra herbacea, erecta, glabra, verisimiliter 3-7 dm. alta, caule ramisque valde tetragonis. Folia tenuiter petiolata petiolis sparsim ciliatis (1-) 2-5 cm. longis, petiolo adjecto 7-12 cm. longa, principalia tripartita, foliolis ovato-lanceolatis vel suboblongo-lanceolatis, membranaceis, glabris et eciliatis, serratis dentibus indurato-apiculatis, lateralibus sessilibus vel parce subsessilibus, omnibus apice acutis vel terminali breviter acuminato. Capitula corymboso-paniculata, numerosa, pedicellata pedicellis tenuibus hispidisque, radiata, pansa ad anthesin circ. 1.3-1.7 cm. lata et circ. 4 mm. alta. Involucrum inferne plus minusve hispidi bracteae exteriores circ. 4-6, lineari-oblongi, patentes, saepius ciliatae, apice obtusae, tantum circ. 1.5-2 (raro - 2.5) mm. longae, quam interiores lanceolato-oblongae apice minute pubescentes dimidio breviores. Flores ligulati plerumque 5, flavi, ligula late oblanceolata, apice minute denticu-

lati. circ 7-9 mm. longi. Achaenia anguste linearia, infra medium recta supra (saltem apicem versus) valde curvata, glaberrima, nitide brunneo-nigra, exaristata, valde obcompressa, non manifeste striata, exalata, circ. 1-1.2 cm. longa et \mp 0.7 mm. lata, demum paleas oblongo-lineares valde superantia.

Specimens examined: *William Bush* 32, alt. 1200 ft, on south slopes in semi-arid valley, Palikea, Isl Oahu, Hawaiian Isls., Jul. 7, 1929 (type. Herb. Field Mus., 2 sheet-⁺ cotypes, Herb. Berl., Herb. Kew).

Offering deceptive resemblances (whence the trivial name) to *Bidens fulvescens* Sherff in texture and color of foliage; to *B. sandwicensis* Less. and *B. conjuncta* Sherff in the herbaceous, acutely tetragonal branches and upper portions of stem, and to *B. conjuncta* further in the sessile lateral leaflets.

***Bidens oligocarpa* sp. nov.**—Herba annua, erecta, gracilis, 4-6 dm. alta, caule ramisque acriter tetragonis et sparsissime hispidis. Folia petiolata petiolis tenuibus ciliatis usque ad 1.5 cm. longis, petiolo adjecto 4-8 cm longa, pinnatim 3-5-partita, foliolis ovatis vel oblongo-lanceolatis, valde membranaceis, acriter serratis dentibus indurato-apiculatis, faciebus sparsim minuteque adpresso-hispidis, marginibus ciliatis, terminali usque ad 2 cm. lato et apice acuminato. Capitula pauca, ramos (pedunculos) tenuissimos usque ad 8 cm. longos terminantia, radiata, pansa ad anthesin circ. 1.2-1.6 cm. lata et 5-7 mm. alta. Involucri subglabri bracteae exteriores 5-7, tenuissime lineares, hispido-ciliatae, supra moderate dilatatae, apice acerrimae, 1.5-2.3 mm. longae; interiores late lanceolatae vel oblongo-ovatae, apice pubescentes, 3-3.5 mm. longae. Flores ligulati 4-5, rosacei, ligula oblongi vel anguste obovati, apice obtuso obsolete denticulati, 7-9 mm. longi. Achaenia circ. 6-8, linearia, obcompressa-tetragona, omnino circ. 8— (unica facie 2—) sulcata, atra, superne sensim angustata, exalata, plus minusve erecto-hispida setis e faciebus ipsis vel e papillis ortis, corpore 6-10.5 mm. longa et 0.7-0.9 mm. lata, apice biaristata aristis retrorsum hamosis, 0.4-1 mm. longis.

Specimens examined: *C. E. Lloyd* 409, State of Sonora, Mexico, 1890 (type in Herb. Gray).

BIDENS SKOTTSBERGII var. **conglutinata** (Deg. & Sherff) comb. nov.; *B. hawaiiensis* var. *conglutinata* Deg. & Sherff, Bot. Gaz.

89:364. 1930.—At the time the var. *conglutinata* was described, *B. skottsbergii* had not been published. A study of the achenial characters now shows that the variety has the achenes of *B. skottsbergii*, and in fact matches that species rather well except that the species has noticeably larger rays.

Ericentrodea decomposita Blake & Sherff sp. nov.—Scandens, verisimiliter fruticosa, caule obtuse angulato moderatim hispido pilis multiloculatis, divaricate ramoso. Folia opposita, principalia divaricata, petiolata petiolis marginaliter dense ciliatis aliter sparsim pilosis (omnibus pilis multiloculatis) 5–7 cm. longis, petiolo adjecto ± 1.5 dm. longa, plerumque quadraternata, segmentis indivisis vel rursus lobatis, segmentis vel lobis ultimis diverse oblongis vel rhomboideis vel lanceolato-ovatis plerumque 1–3-dentatis dentibus apicaliter rotundatis et abrupte mucronulatis, marginibus sparsim ciliatis pilis saepe multiloculatis, facie superiore glabra coriacea, facie inferiore glabrata vel ad costas (praecipue medianam) moderate pilosis pilis elongatis multiloculatisque. Capitula cymoso-paniculata, tenuiter pedicellata pedicellis hispidis circ. 1–2 cm. longis, discoidea, pansa ad anthesin circ. 11–13 mm. lata et circ. 8–9 mm. alta. (ex floribus tubulosis siccis) flava. Involucri hispidi bractee exteriores 8–10, tenuiter lineares vel subulatae, apicaliter acerrimae, 3.5–4.5 mm. longae, interiores lineari-lanceolatas paulo superantes. Paleae oblongo-ovatae, subbrunneae, marginaliter diaphanae, demum circ. 4.5–5 mm. longae. Flores tubulosi ± 35 , subcylindrici, infra non manifeste in tubum contracti, hermaphroditi, corolla circ. 5 mm. longi, limbo 5-dentati; stylorum ramis apice triangulatis, acuminatis, papillois. Achaenia (submatura!) plana, oblanceolata, utrinque truncata, faciebus brunneo-nigris obscure lineata et glabra vel apicem versus raro erecto-setosa, marginibus densissime pectinato-ciliata pilis subbrunneis adscendentibus et longitudine corporis diametrum etiam superantibus, corpore tantum circ. 1.5–2 mm. longa et circ. 0.5 mm. lata, apicem versus non cervicata sed ad apicem ipsius latus quodque plerumque 3–4-aristata (itaque omnino plerumque 6–8-aristata) aristis basaliter rarius connatis, tenuibus, stramineis, 1.5–2 mm. longis, retrorsum hispidis barbatisve.

Specimens examined: Dr. A. Weberbauer 7075, Peru, 1909–1914 (type in Herb. Field Mus.).

In the key to the species of *Ericentrodea* presented some years ago by DR. S. F. BLAKE and myself (Jour. Wash. Acad. Sc. 13:104. 1923), three species were listed: *E. corazonensis* (Hieron.) Blake & Sherff, *E. homogama* (Hieron.) Blake & Sherff, and *E. mirabilis* (Sherff) Blake & Sherff. From *E. corazonensis* the present plant differs at once in having discoid, not radiate, heads and the lower or principal leaves mostly at least quadriternate, not just ternate. From *E. homogama* it differs in having the lower or principal leaves mostly at least quadriternate, not biternate, and in having much narrower ultimate segments to the leaves. From *E. mirabilis* it differs again in its mostly at least quadriternate, not biternate, lower or principal leaves; also in having ∓ 35 -flowered heads about 11-13 mm. wide and about 8-9 mm. tall, not ∓ 12 -flowered heads 3-5 mm. wide and about 4-5 mm. tall; in having exterior involucre bracts narrowly linear (or subulate) and smooth-edged (except for hairs), not linear-lanceolate and not laciniate-ciliate or serrulate; in having the achenial awns definitely arranged in two widely separated groups (one group over each lateral margin), instead of being subregularly awned with awns peripherally and equidistantly arranged at the achenes' apex; etc.

XANTHIUM PENNSYLVANICUM laciniatum Sherff & Shull, var. nov.—Some time before his death the late Mr. F. F. CREVECOEUR sent to PROFESSOR CHARLES A. SHULL of the University of Chicago some anomalous specimens of *Xanthium*. They had been found growing in a clump on loam soil at one end of a corn field belonging to Mr. CREVECOEUR, near Onaga, Kansas, July 19, 1928. Mr. CREVECOEUR wrote, "I have been told by several that they have seen the same form southwest of Onaga, but I have seen only those grown on my farm." Interested in the plants because of their laciniate foliage, he planted burs "in a highly manured garden and no plants exceeded 24 inches in height as guessed at. At any rate, none were 30 inches high." Burs from the CREVECOEUR material were planted by DR. SHULL in the summer of 1930, in the experimental garden of the University of Chicago. There I was very kindly permitted by him to make frequent observations and later to collect herbarium specimens for study and description.

The twenty or more plants raised under our observation were

strikingly uniform¹ in being somewhat dwarfed (only about 4-6 dm. high) and in having the leaves with rather small, deltoid blades (mostly 6-8 cm. wide), these laciniately 3-5-lobed, the sinuses sometimes extending even to the rhachis and the several teeth of each lobe becoming attenuate-linear. The fruits were exactly like those found on normal *Xanthium pennsylvanicum* Wallr.

Numerous specimens were collected and these will be distributed to important herbaria. The technical description follows: Plantae subpumilae, 4-6 dm. altae. Folia principalia petiolata petiolis tenuissimis plerumque 5-9 cm. longis, laminis deltoideis 6-8 cm. latis laciniatim 3-5-lobatis sinibus saepe profundis vel etiam usque ad rhachidem incurrentibus dentibus marginalibus sursum attenuato-linearibus.

Specimens examined: *Earl E. Sherff* 5012, cultivated in experimental garden of University of Chicago, Chicago, Illinois, Oct. 11, 1930 (type in Herb. Field Mus.).

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

¹ One plant in the culture was typical *X. pennsylvanicum* in all respects, but I assume that this had resulted from an admixture of burs from other stocks that had been used in making certain comparisons.

EMBRYOLOGY OF OSMUNDA CINNAMOMEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 422

GEORGE L. CROSS

(WITH EIGHT FIGURES)

Introduction

The external appearance, anatomy, and meristems of the Osmundaceae have been shown to represent a condition intermediate between the eusporangiate and leptosporangiate ferns, which led to the suspicion that the embryology might likewise show intermediate characteristics. Data of this kind should be of value in studies of classification and phylogeny. The uniformity of structure of the embryo of the more recent ferns is entirely lacking in those which are primitive. In some of the latter a suspensor is present, a feature not seen in recent ferns, although common in lycopods and seed plants.

The desirability of classifying ferns as eusporangiate and leptosporangiate is probably more apparent than real, but for want of something better such a classification still persists in the literature. To distinguish between the two groups, technicalities beyond the purpose of this paper are involved. BOWER states that eusporangiate ferns usually have more than a single apical cell involved in their meristems, that is, they usually have a group of master cells involved in growth at the stem tip, root tip, and in the production of sporangia. In the case of the leptosporangiate forms, practically all structures can be traced in origin to the activities of a single apical cell, with definite segmentation. There are also certain embryological differences which will be considered more in detail.

Although not all the families of the Filicales have been investigated embryologically, the embryo of a typical eusporangiate fern, such as *Marattia*, has been described by CAMPBELL (3) as follows:

The first division of the embryo is perpendicular to the archegonium and parallel to the surface of the gametophyte. It divides the embryo into epibasal and hypobasal halves. Further divisions result in a quadrant stage, an octant stage, and finally a somewhat unsymmetrical mass of tissue in which the ap-

pendages are not clearly distinguishable as to their origin. Ultimately leaf, stem, and root are produced from the epibasal half of the embryo, while the entire hypobasal half is given over to the production of a massive foot

In some groups a suspensor is produced, as described by LAND (7) for *Angiopteris erecta*. It is formed next to the neck of the archegonium, and by its enlargement pushes the embryo deep into the nutritive tissue of the gametophyte. The sporeling grows directly upward through the gametophyte.

The embryos of representative leptosporangiate ferns have been described by GOEBEL (5), LEITGE (8), ATKINSON (1), SHAW (10), RAUWENHOFF (9), JANCZEWSKI (6), and others. The ordinary method of development is somewhat as follows: the first, or basal wall is laid down parallel to the longitudinal axis of the archegonium and perpendicularly to the anterior-posterior axis of the gametophyte. It divides the embryo into posterior and anterior halves. The second wall is laid down perpendicularly to the archegonium, but parallel to the surface of the gametophyte, producing the quadrant stage. It is at this stage that the position of leaf, stem, root, and foot are presumably determined. Each cell of the quadrant divides in such a manner as to outline definitely the apical cell of the leaf in the lower anterior quadrant; the stem in the upper anterior quadrant; the root in the lower posterior quadrant; and the foot in the upper posterior quadrant. Divisions in the foot proceed irregularly, however, and the wedge-shaped cell soon loses its individuality. As a result of the position of these primary organs, the young sporeling grows along under the gametophyte and usually comes up through the notch at the apex of the gametophyte.

Since the Osmundaceae have been placed taxonomically with the Leptosporangiates, it would be reasonable to suspect that the embryology would be similar to that of the typical leptosporangiate fern. The earliest and most frequently quoted work on the Osmundaceae was done by CAMPBELL (4) in 1892. The results of his findings with respect to the embryo are substantially as follows: The first wall of the embryo is laid down in a plane parallel with the main axis of the archegonium, presumably perpendicular to the anterior-posterior axis of the gametophyte, although this point is not made clear. The second wall is laid down parallel with the archegonium, and also parallel with the surface of the gametophyte. The embryo at this

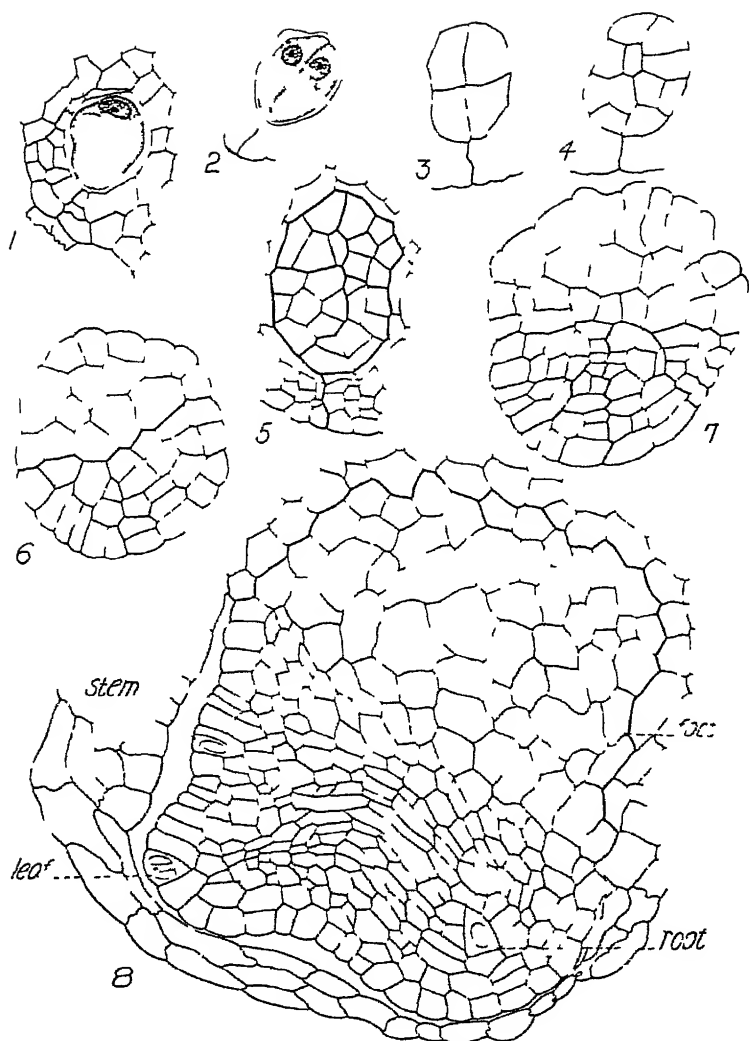
stage is composed of two anterior and two posterior quadrants. The third cell generation results in the production of the apical cell of the leaf in the lower anterior quadrant; the stem in the upper anterior quadrant, the root in the lower posterior quadrant; and the foot in the upper posterior quadrant. The foot becomes massive in the course of its development, a feature which suggests relationship with the Eusporangiates. The embryo usually grows out toward the side of the gametophyte, transverse to the main axis rather than parallel with it, although CAMPBELL does not make any special point of this fact. Obviously, according to CAMPBELL, the method of development is essentially that of a leptosporangiate fern, the main differences being the placement of the second wall parallel with the archegonium and the more massive foot.

Methods

Prothallia of *Osmunda cinnamomea* grown in the greenhouse and in the laboratory produced antheridia as early as the 6- or 8-celled stage, but owing probably to nutritive relationships, the production of archegonia was delayed until the prothallia measured 3-5 mm. in width. Various methods of killing and fixing were employed, but the best results were obtained with chromo-acetic acid in strengths of 0.5-1 per cent of each. Since the young sporophytes grow out at right angles to the gametophyte, sections were cut perpendicular to the anterior-posterior axis of the latter. Sections 8-10 μ were found to be most satisfactory.

Results

Fertilization in *Osmunda cinnamomea* occurs in the usual manner. In all the cases examined the first wall of the zygote was laid down parallel to the longitudinal axis of the archegonium, and parallel to, or at a variable angle with, the anterior-posterior axis of the gametophyte (fig. 2), instead of perpendicular to such axis as indicated by CAMPBELL (4). The second wall apparently may be placed either parallel or perpendicular to the archegonium (fig. 3). CAMPBELL states that it is at this stage that the point of origin of the primary organs is determined, that is, the stem and leaf from the anterior quadrants, and the foot and root from the posterior ones. It is not made clear, however, just how a quadrant stage arises from the placement of two walls, both parallel to the longitudinal axis of the archegonium.



FIGS 1-8.*—Fig 1 fertilized megagamete which has elongated and become vacuolate. Fig 2, first wall of embryo; section parallel to archegonium and perpendicular to main axis of gametophyte. Fig 3, quadrant stage, parallel to archegonium and perpendicular to main axis of gametophyte. Fig 4, embryo just past octant stage, showing rectangular cells not related to origin of apical cells. Fig 5, young embryo. Fig 6, embryo showing origin of foot in upper half. Fig 7, embryo with foot clearly delineated above and possible origin of leaf and stem in lower left portion, section parallel to archegonium and perpendicular to gametophyte. Fig 8, embryo showing foot, leaf, stem, and root; section parallel to archegonium and perpendicular to gametophyte.

* All figures drawn at level of table with Spencer camera lucida, under 4 mm. Leitz apochromatic objective, with periplan ocular 8X, reduced to one fourth original size.

To obtain a median section of such a quadrant, one would cut sections of the embryo in a plane perpendicular to the archegonium, in which case it would seem also that the appendages would arise in a plane perpendicular to the archegonium. It would preclude the possibility of obtaining longitudinal sections of the embryo in any but sections perpendicular to the archegonium, a concept not in accord with CAMPBELL's figs. 95 and 96, which show longitudinal sections of embryos in such a manner that a longitudinal view of the neck of the archegonium is also presented.

I was able to obtain the best longitudinal sections of embryos by orienting the thallus with its longitudinal axis perpendicular to the knife. This method showed that the quadrants had no fixed orientation with respect to the thallus, and, as will be shown later, specific quadrants are not correlated with the ultimate position of the primary organs.

Sometimes there is delay in formation of the walls of the embryo, and the embryo elongates along the major axis of the archegonium in such a manner as to suggest a suspensor (fig. 2). In such cases the nucleus migrates to the proximal end of the embryo, and the distal end becomes highly vacuolated. The vacuolated portion may be regarded as a rudimentary suspensor, although the situation occurs infrequently, and studies were correspondingly limited. If two megagametes on the same thallus are fertilized, the younger of the resulting embryos frequently passes through such stages, probably owing to scarcity of food. This tendency toward suspensor formation, if it may be regarded as such, is certainly suggestive of the eusporangiate ferns.

The quadrant stage is succeeded by the octant stage through the medium of a third generation of cell walls, four in number, which divide each of the quadrants into two octants, one of the latter being triangular in profile, the other rectangular, as described by CAMPBELL (4). The triangular octants resemble apical cells, but the same situation prevails in *Riccia*, *Marchantia*, and other forms with spherical embryos; and as such views may be obtained by cutting through the center of the embryo in two different planes, it does not follow that they are apical cells. In fact, subsequent divisions rapidly obscure the individuality of these cells, and a relatively large globular mass of tissue is produced, in which the foot is the only appendage

clearly discernible (figs. 6, 7). As may be seen by reference to these figures, the foot is distinct as such at a relatively early period in ontogeny, the line of demarcation between it and the rest of the embryo apparently being the second wall laid down in the zygote. Thus it seems that the foot arises from the entire half of the embryo next to the gametophyte, instead of from the upper posterior quadrant as described by CAMPBELL (4); although the origin of appendages may not be infallibly ascribed to definite halves, quadrants, or octants of the embryo. Probably a better concept of the situation is that the foot arises roughly from that half of the globular mass of tissue next to the gametophyte; and leaf, stem, and root arise from the half of the embryo abutting the neck of the archegonium (fig. 8).

The cells of the foot undergo enlargement, and to a limited extent division. The region thus keeps pace with the more actively dividing mass of cells to its exterior, with the result that the embryo retains, to some extent, its spherical shape, even though a relatively large mass of tissue is produced (fig. 7). This feature, of course, is much more suggestive of the eusporangiate than of the leptosporangiate ferns. The decline of cell divisions in the foot may be correlated with an extensive conduction of nutrient materials to the meristematic tissues beyond, a condition which in turn probably interferes with normal nuclear phenomena, and is facilitated by the large size of the cells involved. The rudimentary conductive system thus arising is considered by LAND to be the primary root of the embryo.

Fig. 7 represents what may be the origin of the leaf and stem apices from the upper distal quadrant, the root from the lower distal quadrant, and the foot from the proximal half of the embryo; but many sections in corroboration of this interpretation were not obtained. Some forty embryos were sectioned, and a preponderance of evidence favored an indeterminate origin of all appendages. This is in correlation with the situation found in the eusporangiate ferns. The suggestion of a suspensor, either extant in the past or to manifest itself in the future, still further suggests the latter group.

The epidermal and hypodermal layers of cells about the portion of the embryo next the calyptra, especially the region which is destined to give rise to the root, become thick walled and filled with tannins and oils. Interruptions in this protective thickened layer occur only at the stem and leaf apices, where mucilage is secreted to

prevent desiccation after the young plant has ruptured the calyptra. The tannin and oil laden cells, adjacent to the apical cell of the young root, act first as a protection to the root in its efforts to break the calyptra, and later become part of the root cap.

The apical cell of the stem arises from an epidermal cell of the embryo (fig. 8). It is usually about three times as long as it is broad, tetrahedral in shape, and of irregular segmentation.

The leaf initial arises usually very close to the stem tip, possibly from the same quadrant as suggested above (fig. 7). It is a triangular pyramidal apical cell with regular segmentation, in contrast to the condition found in most leptosporangate forms, where a 2-sided apical cell is the rule. It is always oriented with one of the flat sides toward the stem tip, a situation reported by BOWER (2) for leaves that are formed later. The segments derived from the two sides of the apical cell away from the stem tip divide more rapidly than the ones from the third, so the leaf is circinate.

The root initial arises endogenously in the same half of the embryo as the leaf and stem initials, instead of exogenously in the region described by CAMPBELL (4). It is first noticeable as a rectangular cell, difficult to distinguish from its neighbors until it has cut off one or two segments (fig. 8). It is usually somewhat enlarged, with transparent contents. It may take the form of a triangular pyramidal apical cell with four cutting faces, the usual case, or it may be a 4-sided pyramid with five cutting faces. The inner half of each lateral derivative of the apical cell contributes to the vascular system of the root, the outer half to the cortex. Shortly before rupture of the calyptra, the vascular system of the young embryo is outlined, continuously from the root tip to the first leaf, with the suggestion of a divergence of a portion of the strand to the second leaf (fig. 8).

According to CAMPBELL, the first leaf ruptures the calyptra by its growth. Studies of sections such as are represented by fig. 8 indicate that the calyptra, which has been distended and flattened by the growth of the embryo as a whole, is ultimately ruptured by the first soil root. The latter grows rapidly and penetrates the substratum before the first leaf reaches any appreciable size.

Summary

1. The first wall in the embryo of *Osmunda* is laid down in a plane parallel with the longitudinal axis of the archegonium, usually paral-

lel with the longitudinal axis of the thallus. The second wall is laid down parallel or perpendicular to the archegonium, forming a typical quadrant stage. An octant stage follows.

2. The origin of the primary appendages cannot be referred to definite halves, quadrants, or octants of the embryo.

3. The primary leaf, stem, and root are produced from approximately one-half of the embryo adjacent to the neck of the archegonium. The foot consists of the half of the embryo next the gametophyte.

4. The root is endogenous in origin.

5. The preceding features, when considered collectively, indicate that *Osmunda* stands nearer the eusporangiate than the leptosporangiate level when embryological evidence is considered. The two main points in common with the eusporangiate ferns are: (1) the origin of the leaf, stem, and root from the same half of the embryo, and the foot from the other half; (2) the occasional presence of an incipient suspensor. The contrasting points are: (1) the placement of the first wall with respect to the axis of the archegonium; (2) the direction of growth of the embryo. It seems logical to assume that the Osmundaceae may be intermediate between the eusporangiate and the leptosporangiate ferns with respect to embryological features.

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[Accepted for publication January 28, 1931]

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DEVELOPMENT OF SEX ORGANS OF FERN PROTHALLIA UNDER PROLONGED CULTIVATION

DAVID M. MOTTIER

(WITH TWO FIGURES)

Certain vigorous fern prothallia were transplanted to culture dishes upon rich soil and given plenty of space, and have been kept successfully in continuous growth for eight years. The plants themselves, or their continuation shoots (clones), have attained the size, and in general outline the appearance, of the liverwort *Marchantia* (fig. 1). Four years after the experiment was begun the results were published in this journal (3, 4). The cultures have now been grown uninterruptedly for eight years, and thus the continuation shoots have been kept under constant observation. It would be possible to continue the cultivation indefinitely, if the difficulties encountered could be overcome.

In former publications the method of cultivation was described somewhat in detail, the difficulties which arose from time to time were mentioned, and remedies given. The most dangerous and annoying feature was the presence of the larvae of a small gnatlike dipterous insect (4). Fumigation of the greenhouse gave only temporary relief, for the gnats doubtless came from outside sources. They and their maggots appeared again in a comparatively short time, and it seemed that the cultures were doomed. Not only that, but new cultures started from spores were at times completely destroyed. In due time, however, and before the continuation cultures were seriously injured, an effective remedy was found which consisted in fumigating the cultures in which the larvae were found with the vapor of carbon bisulphide. When this is applied properly the larvae are killed in a short time without injury to the plants. The method consists in placing a little carbon bisulphide in a small stender dish under the bell glass covering the culture. For a 5×9 inch bell glass about 1.5 cc. of the carbon bisulphide is sufficient. If larger doses are given, the plants are likely to be injured or killed.

The fluid soon evaporates, and after two or three hours the bell glass may be lifted for more complete ventilation. Carbon bisulphide is an effective and convenient fumigant because it vaporizes at low temperatures, and because the lethal dose for both pest and plant may easily be ascertained by trial.

A vigorously growing continuation shoot, if placed upon rich soil (preferably woods earth), may result in the growth shown in fig. 1. In form and size this figure shows the large liverwort-like branches with their massive midribs and ruffled margins. In many shoots, as was shown in a former publication, the branching is typically dichotomous. In the lower right hand corner of fig. 1 a portion has become detached from the parent system of shoots through death and decay of the oldest portion. It often happens that the plants so develop that the shoots become crowded and overlap (fig. 2). Owing to this overlapping some of the shoots become distorted, some growing upward into the air and developing numerous brown rhizoids from the upper as well as from the lower surface of the midrib. Archegonia are sometimes developed from both surfaces in such shoots, as well as upon those spread out flat, as in fig. 1.

Two other features of behavior appearing especially, although not exclusively, in the larger shoots are usually to be observed. They consist in the development of small proliferations springing from the surface of the midrib, from near the margins, or directly from the edges of the margins. Some of those from the margins are thin and irregular in outline, suggesting the tiny pinkish scales which develop from the margin of the thallus of *Marchantia*. These marginal scalelike proliferations are usually but one cell in thickness, and they are frequently white in color owing to lack of chloroplast development. Eventually they wither and dry up. They do not remain flat as they develop, but become rolled or folded.

The second kind of minute proliferation mentioned bears numerous antheridia. The antheridia-bearing proliferations arise from the midrib or from the wings of the thallus. It will be recalled that one of the important conditions necessary for continued growth of the prothallia or their clones is prevention of the development of embryo sporophytes. This can easily be accomplished if the plants are watered by sub-irrigation. In large and robust shoots no great



FIGS. 1, 2.—Fig. 1 (left), clones of prothallia of *Madenecia nodulosa* grown without appreciable crowding; fig. 2, culture of prothallial clones of same species which have become crowded in growth; slightly reduced

care is necessary, since upon them only archegonia are produced. The entire tendency is toward femaleness. I have not found antheridia upon the midribs or the massive parts of such plants as are shown in fig. 1. On the contrary, when growth is less vigorous the small antheridia-bearing proliferations will appear. They may be numerous upon the upper side of older midribs which have begun to darken or to turn brown. To the unaided eye, or when viewed with the aid of a pocket lens, these antheridial outgrowths appear as yellowish-green granulations. They are literally covered with antheridia, the production of which exhausts these proliferations.

The most vigorous and rapidly growing specimens were, as a rule, purely female, antheridial outgrowths appearing upon those which were crowded or upon the older and doubtless more poorly nourished parts. These facts seem to lend strongest support to the theory that sex is purely quantitative and not qualitative. There is certainly no sex chromosome.

It is unnecessary to discuss the constantly increasing evidence concerning sex as a quantitative phenomenon in inheritance. The following comments will be confined to the prothallia of the species under consideration, *Matteuccia nodulosa* (*Onoclea struthiopteris*). For many years the writer has grown annually prothallia of this plant in many pure cultures. Careful observation of the spores shows that they vary in size, and, even when sown under the most favorable growth conditions, some of the spores give rise to plants of exceptional vigor, while other spores produce plants which are much less vigorous from the start. The larger and more robust plants develop into pure females, while the smaller ones may begin to bear antheridia when they are very minute, and continue to do so to the point of exhaustion, or to complete cessation of growth. It seems that in such plants the stimulus to develop antheridia far exceeds the stimulus leading to multiplication of vegetative cells. Of the large female specimens, that is, those bearing archegonia, only an occasional one produced a few antheridia upon the older parts. From a most critical study it was found that not more than 10 per cent of these large plants bore antheridia before the time of production of archegonia, and in many cases but one or two antheridia appeared. The cultures of the past eight years have shown that

large and vigorously growing plants developed only archegonia, and that when antheridial proliferations appeared they were found to develop from the older parts which were soon to die. When antheridia appeared upon the more thrifty plants, these sex organs were produced upon the weaker parts or upon the thinner marginal lobes. It seems reasonable to conclude that the more vigorous plants tend to remain female, and that production of antheridia is associated with less vigorous growth. Whether diminished vigor is the result of poorer nutrition in these plants, the writer does not know.

This study began eight years ago with the prothallia of two species, *Matteuccia nodulosa* and *Osmunda claytoniana*. During the past four years the cultures of *O. claytoniana* became weak, the majority dying; only a few clones now survive, and their growth is slow. In the earlier years these prothallia produced good vigorous cultures. Near the close of the fourth year, however, when prothallia were examined histologically, they were found to contain an endophytic fungus (4). Whether this endophyte contributed to the weakening of the plants the writer is unable to say. Endophytic fungi were not observed in prothallia of *Matteuccia*.

Summary

1. The continuation shoots (clones) of individual prothallia of *Matteuccia nodulosa* and *Osmunda claytoniana* have been grown in cultures for eight years (1922-1930).
2. Their continued existence was made possible by preventing the development of sporophytes. All conclusions reached at the end of the first four years of continuous cultivation were confirmed during the subsequent four years of observation.
3. The vigorous clones attained the size of the liverwort *Marchantia*, having a pronounced midrib and branching dichotomously.
4. When growth was most vigorous and rapid the clones bore, as a rule, only archegonia. Less favorable conditions of growth facilitated the production of antheridial proliferations. These developed mainly from the older parts of the midrib and from the thinner margins. The proliferations seem to be exhausted in the production of antheridia, as they did not continue to grow, but finally dried up.
5. This study lends unquestioned support to the theory that sex

is quantitative and not qualitative. The spores and their resulting gametophytes are bisexual haploids.

6. Vigorous growth results in the development of archegonia almost exclusively, while less vigorous growth leads to the production of antheridial proliferations also upon the same clones.

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[Accepted for publication December 19, 1930]

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CURRENT LITERATURE

BOOK REVIEW

International address list of botanists

One of the most important concrete results of the Fifth International Botanical Congress at Cambridge in 1930 was the decision to arrange for the publication of an international address list of botanists, to take the place of DÖRFLER's *Botaniker Adressbuch*, which served the botanical world so well, but is now long out of date. The new address list will be on somewhat similar lines to DÖRFLER's book and will contain the names of some 13,000-14,000 botanists and botanical institutions in all parts of the world. These will be arranged alphabetically by countries, and in most cases will be printed in the language of the country and will be provided with an index of personal entries and geographical indices. The price of the book is \$3.25, the low cost being possible owing to the assistance received from the Bentham Trustees and the Carnegie Institution of New York. Advance orders are now being taken by Messrs. Bailliere, Tindall & Cox, 7 & 8 Henrietta Street, Covent Garden, London WC 2.

THE
BOTANICAL GAZETTE

November 1931

NOTES ON THE GENUS *LEMANEA*
IN NORTH AMERICAGEORGE F. ATKINSON¹

No adequate knowledge of the structure, development, and relationship of the genus *Lemanea* existed until the appearance of SIRODOT's classic work in 1872.² Prior to this time the knowledge of species rested almost entirely on superficial characters. The few studies of internal structure were so incomplete as to add diagnostic features of very little value. In a number of cases, forms representing distinct sections of the genus were placed in the same species, sometimes even mixed in a single collection, or packet. Since SIRODOT's work, there is no longer any difficulty in recognizing the sec-

¹ After the publication of the monograph of *Lemanea* in 1890 (ATKINSON, GEO. F., Monograph of the Lemnaceae of the United States. Ann. Botany 4: 177-229. 1890), Professor ATKINSON received a number of specimens of this group from several parts of North America. During the two years preceding his death in 1918, he devoted considerable time to a study of this material of *Lemanea*. He also studied most of the available material in North America of which he could learn by correspondence with institutions and collectors whose herbaria he thought probably contained collections of this genus. A number of individuals contributed specimens or permitted the retention of a small portion from the packets for his study. All this material, together with Professor ATKINSON's notes on *Lemanea*, are deposited in the Cornell University herbarium, in which all the known species of North America are thus represented.

It is to be regretted that Professor ATKINSON's untimely death prevented the publication, as he had planned, of his notes on the American species of *Lemanea* and their

[Footnote 1 continued on following page]

² SIRODOT, S., Étude anatomique, organogénique et physiologique sur les Algues d'eau douce de la Famille des Lémanées. Ann. Sci. Nat. Bot. Ser. V. 16: 1-95. Pls. 1-8. 1872.

tion of the genus to which any specimen belongs, provided one takes the little trouble necessary in determining the one feature in the internal structure of the sexual shoots which clearly distinguishes the two sections of the genus.

The species are remarkable for their peculiar habitat, as well as for the season of the year during which they begin and complete their growth. They live only in fresh water, where the water is turbulent, as in swift streams, at waterfalls or rapids, or especially on the downstream side of large stones in rather deep, strong-flowing streams. Rarely do they occur in sluggish river water. The specimens occur on the edge of rock ledges in rapids or on the rock surface at the foot of waterfalls where the water often strikes with great force, or on the eddying side of boulders or rocks.

The species grow only during the cold months of the year. Sometimes in mountain rivulets the winter growth may be retarded, so that maturity may not be reached in the icy water until sometime in the summer. In some regions, where the autumn season is warm, the cold weather for beginning of growth may not arrive until winter is well advanced, so that maturity may not be reached until late spring. But in the streams in the region of Ithaca, New York and in central North and South Carolina, in fact generally throughout

distribution. These notes, which were practically ready for publication, have been edited with the hope of making available the results of his intimate knowledge and study of this group of interesting algae.

A number of the specimens which Professor ATKINSON studied, especially those in the Cornell University herbarium, are indicated in the text by letters. This does not necessarily mean that the same species or duplicates of a given number may not be represented in another herbarium, nor are all the collections of any one herbarium indicated. The herbaria are indicated as follows:

C, Cornell University
Calif., University of California
F. W.G. Farlow
M, University of Michigan
N.Y., New York Botanical Garden
U.S., United States National Museum
Y, Yale University

While certain changes and omissions have been made, the manuscript remains substantially as prepared by Professor ATKINSON. Yet it would be unjust to hold Professor ATKINSON responsible for any discrepancies that may appear. Despite the effort to retain his style and individuality, the quality of the notes is no doubt altered by passing through the hands of another.—W. C. MUENSCHER.

their range, the species reach maturity during late winter or early spring. They thrive, therefore, in water very near the freezing point, often under ice. From this period on, the sexual shoots, by weathering and whipping in the strong current, become more and more broken, while surface features in the characteristic antheridial zone are worn down. For this reason, specimens gathered during the summer, the usual season for such collections, have frequently lost so many distinctive features that *specific* determination is often rendered extremely uncertain.

The spores germinate in late autumn or early winter, and form a branched filamentous or cellular sole which gives rise to the erect, branched, *Chantransia*-like but sterile protonemata. The sexual shoots arise as branches from the protonemata, either near the base, along the middle, or in the superior parts. The *Chantransia*-like protonema gives rise to rhizoids, usually near the base, but also along the middle region. These rhizoids add to the sole on the surface of the rock. Rhizoids also arise from near the base of the sexual shoots, and contribute also to the sole. The rhizoids also produce *Chantransia*-like protonemata and even sexual shoots. It is interesting to note that the interrelations and functions of the sole, the *Chantransia*-like protonemata, and the sexual shoots parallel remarkably the interrelations and functions of the protonemata and leafy stems (sexual shoots) of the mosses.

The branch which forms the sexual shoot is much stouter, even at its origin, than are the sterile branches. It grows by a single apical cell, from which short, tabular cells are cut off behind by cross walls, in regular succession. The apical cell is short and convex. The tabular cells are at first broader than long. Each tabular cell develops into a definite morphological and structural unit of the sexual shoot. The sexual shoot consists of "nodes" and "internodes." The shoots vary in length from 20 up to 200 or more internodes. The nodes are short, and in most species are more or less prominent because of their greater diameter than that of the internodes. The internodes are comparatively long, three to six times longer than their diameter, are either cylindrical, slightly constricted, or shaped like an hour-glass. The sexual shoots, therefore, present a more or less "nodulose" or "torulose" appearance, whence the specific names of *nodosa*, *torulosa*, *catenata*, etc.

Each tabular cell develops into a definite morphological and structural unit. A single node is an antheridial zone, the antheridia being massed in definite fields forming a whorl, or in a ring over its surface. A single internode is a procarp zone, since the procarp branches are distributed throughout the length of the internode, or are limited to the middle area or to the ends; that is, to the portions of the internodes adjoining the nodes. The morphological or structural unit, which is developed from a single tabular cell of the young sexual shoot, consists of a single internode and the adjacent halves of each adjoining node. Description of the successive stages in the development of one of these units of the sexual shoots is too complicated, and would require too much detail, for the purpose of this paper. It is in brief a process of cell division, combined with branching.

The terminal branches remain closely united, forming the three to five or more cell-layered wall of the shoot. The internal, or primary, branches elongate rapidly and undergo no further branching (with the special exceptions in one section of the genus). The result is a hollow sexual shoot with four primary branches arranged cruciately. They stand at the middle of each internode. There is a central axis consisting of a single row of greatly elongated cells, one cell for each "morphological unit" of the shoot. The elongation of this cell is downward into the adjacent morphological unit, reaching nearly to the middle of the latter, where it joins the corresponding cell of that unit. This central axis cell then bears the four primary ray cells near its apex or distal end, but in the middle of its corresponding procarp zone.

In one group of species (*Sacheria*) these ray cells are L- or T-shaped, the arm or arms of the L or T being closely applied to the wall of the sexual shoot. In the other group (*Eulemanea*) the ray cells are straight, and the distal end of each is separated from the wall by an oval or pyriform cell. From the distal ends of the ray cells, in both groups of species, two to four secondary branches arise, some extending upward, some downward. These secondary branches are long and consist of numerous cells placed end to end. In *Sacheria* they lie close to the wall throughout their entire length, while in *Eulemanea* they are separated from it by oval or pyriform cells, at regular intervals, except near the antheridial zone. On their outer

surface these secondary (or in *Eulemanea* tertiary) branches give rise to more numerous short branches, which themselves branch rapidly several times, forming the cellular wall. The inner cells of the wall are larger and fewer in number; the outer ones smaller and more numerous. The outer portion of the wall consists, in some species, of one layer and, in other species, of two layers of small short cells forming a compact palisade. The homologous outer layers of cells, in certain definite areas on the antheridial zone, are the antheridia, each of which contains a short, stout sperm cell, or "spermatium." The antheridia, therefore, form a complex or group of cells, terminating the secondary branches which meet in the antheridial zone from the two adjacent procarp zones. The cortex of the shoot is formed from the tips of the lateral branches arising from the secondary branches.

These secondary branches, which line the inner surface of the wall, give rise also to the procarp branches. Each procarp consists of a single row of four to ten short cells. The terminal one to three cells lie in the wall, while the trichogyne from the carpogone extends to the surface. The secondary branches, which bear the procarps, were termed by SIRODOT "tubes lateraux," by ATKINSON "generative filaments," by OLTMANNS "Längsfäden." After fertilization, the carpogone, or fertilized egg, gives rise to the short ooblastema filaments which bear the branched spore chains, forming the cluster known as the cystocarp. The growth direction of the spore chains is toward the center and in the cavity of the sexual shoot, which becomes more or less filled with carpospores, according to the species or the varying number and size of the cystocarps.

One interesting and important morphological feature, characteristic of certain species, remains to be described. This is the envelopment of the central axis of the sexual shoot by numerous slender threads which arise near the proximal ends of the four primary branches or rays. In all the species investigated, with one exception, these enveloping filaments arise from the under side of the rays, and extend downward in a spiral manner, winding around the central axis, often forming a thick and dense covering. The one known exception is *Lemanea parvula* Sirodot, in France. In this species, according to SIRODOT, the enveloping filaments extend upward.

SIRODOL revised the genus *Lemanea* and recognized two genera. He retained the genus name *Lemanea* for those species in which the enveloping axial filaments from the ray cells are present. For the other group of species, devoid of enveloping axial filaments, and which he considered to be of generic rank, he gave the name *Sacheria* (Ann. Sci. Nat. Bot. Ser. V. 16:70. 1872). Other characters are correlated with this fundamental one of the presence or absence of filaments enveloping the central axis. Some of them appear to be constant; for example, the forms of the ray cells and the relation of the generative filaments to the wall. Others are variable in certain species; for example, in the *Sacheria* section procarps usually arise in and near the antheridial zone, but in *Lemanea* (*Sacheria*) *fluvialis* they are distributed throughout the procarp zone also. In the *Eulemanea* section the procarps arise in the middle of the procarp zone, and are usually absent from the region of the antheridial zone. But in *Lemanea pleocarpa* Atkinson they are distributed throughout the procarp zone and occur also in the antheridial zone. In the *Sacheria* section, the antheridial zones are usually more or less warty and the antheridia are confined to these protuberances; while in the *Eulemanea* section the antheridial zone is usually even and the antheridia are usually associated in a broader, narrow, encircling band; but variations occur.

In the *Sacheria* section the procarp branch is short (two to four cells) because the generative filaments lie close to the inner surface of the wall throughout their entire length. In the *Eulemanea* section the procarp branch is longer (five to ten cells) because the generative filaments are separated from the wall by stout cells except in the antheridial region. In the *Sacheria* section the hypogynous cells (those below the egg cell) do not proliferate; they thus remain naked except as they are covered by the group of carpospores. In the *Eulemanea* section many of the hypogynous cells of the procarp proliferate, producing short filaments. This proliferation of the hypogynous cells of the procarp suggests a primitive cystocarp wall. In the *Eulemanea* section proliferation takes place also in the inner wall cells of some species.³

³ In the monograph of the Lemnaceae of the United States, ATKINSON, while recognizing the great merit of SIRODOL's work, employed only the genus *Lemanea*, treating *Sacheria* as a subgenus. This procedure was followed by subsequent authors. In the present notes *Sacheria* is treated as a section of *Lemanea*.

SECTION EULEMANEA

LEMANEA ANNULATA Kütz. Phyc. Germ. 261. 1845; Species Alg. 528. 1849; Tab. Phyc. 7:33. pl. 84, fig. 1. 1857.

When the spores are mature, *L. annulata* and *L. fluviatilis* present a close superficial resemblance, and they are apt to be confused unless the internal structure is examined. The difference is then very apparent since they belong to distinct subdivisions of the genus. In *L. annulata* the antheridial zone is light in color, as in the young stages, but the middle of the procarp zone is dark from the mass of spores. *L. fluviatilis*, when the spores are mature, presents alternating light and dark zones, but since the spores are borne next to the antheridial zone, and not in the middle of the procarp zone as in *L. annulata*, the antheridial zone is dark, while the middle of the procarp zone is light, since the violet color fades from the walls at maturity.

California: in rapidly flowing water on the rocky bed of a small stream which becomes dry in the summer, hills about 2 miles southwest of Bloomington, San Bernardino Co., *S. B. Parish*, Mar. 23, 1897, Phyc. Bor. Am. no. 329 (C); in stream Tamalpais, Marion Co., *E. L. Greene*, May, 1886 (Calif.); Corkscrew Falls, near Bluff Lake, San Bernardino Mts., altitude 7400 ft., *S. B. Parish*, June 21-27, 1895 (Calif.); on rocks in swift stream, Mt. Tamalpais, Marion Co., *W. J. V. Osterhout*, July, 1906; in stream, San Luis Obispo, from *Herb. J. W. Bailey* (Calif.); in stream, Santa Margarita, *C. C. Parry*, on Mexican Boundary Survey, Apr., 1850 (N.Y.); in stream, Santa Cruz, *C. L. Anderson*, no date (N. Y.); in stream, Oakland, *C. L. Anderson*, no date (F); streams in vicinity of San Francisco, *T. S. Brandegee* (C); in stream, Univ. Calif., Berkeley, 1871, *Herb., Wölle* as "*L. fluviatilis*" (C); in running water, waterworks of Leland Stanford Jr. University, *N. L. Gardner*, Apr. 19, 1916 (Calif.).

Indiana: Eel River Falls, Owen Co., *L. M. Underwood*, May, 1883, Phyc. Bor. Am. no. 237 (C). This material was formerly determined as *L. catenata* but a re-examination shows that it is *L. annulata*.

Nevada: in stream, Diamond Mts., *Sereno Watson*, U.S. Geol. Exp. 40th Parallel, no. 1546 (Yale).

Oregon: in streams, Oregon City, *A. S. Foster* (F); in streams, eastern Oregon, *W. C. Cusick*, 1886, no. 1353 (F).

Washington: in springs near Rock Creek, Spokane Co., altitude 670 m, *J. H. Sandberg* and *J. B. Leiber*, June 1, 1893 (U S.); (C).

LEMNEA ANNULATA Kütz. var. *franciscana* Atkinson, var. n.

Sexual shoots slender, 10–20 cm. long, less than 1 mm. in diameter, straight, very pliant, dark violet, blackening when dry, tapering gradually at the base. Procarp zones short, tapering slightly and gradually from the antheridial zone and thus nearly cylindrical. Antheridial band usually regular, but tissue beneath distinctly darker than adjacent wall of procarp zone even at time of anthesis, thus not agreeing with the normal conditions of the species; procarps in middle of procarp zone, the spores even from young stage of the cystocarp giving a darker color to middle portion of procarp zone.⁴

This plant appears to be only a slender and darker-violet variety of *L. annulata*, owing to the habitat of shade and swiftly running water. The darker color of the antheridial band in the young condition violates one of the principal characters of the species. Even in age, when the color fades out greatly from the wall of the sexual shoots, so that a light-colored zone alternates with the middle portion of the procarp zone, darkened by the mass of spores, the antheridial band is still darker in color than the adjacent tissues. But the subadjacent tissue has the same appearance as in normal specimens, forming a slightly depressed ring. This same appearance is sometimes present in large and normal specimens. Even in the older specimens the spores are not mature, since the tips of the chains are slender.

California: in falls in deep, shady ravine, no. 94471 (Calif.).

LEMNEA AUSTRALIS Atkinson. Ann. Botany 4:218, figs 19–44, 47. 1890.

The type material of this species is that collected by *Atkinson* in Morgan's Creek, North Carolina. The material was collected

⁴ To conform with the International Rules of Plant Nomenclature the description is here Latinized: *Stirpes sexuales tenues, 10–20 cm. longae, diametro minores quam 1 mm., rectae, valde flexibiles, atro-violaceae, ad basim sensim angustatae, demum exsiccatae nigrescentes. Procarpiorum zonae breves, paulo sensimque e zona antheridiali attenuatae, quam ob rem fere cylindricae. Vitta antheridialis plerumque regularis, sed caro infra quam murus proximus zonae procarpiorum (etiam ad anthesin) manifeste fuscior, itaque condicionibus normalibus pro specie non congruens; procarpiis ad medio suae zonae positis, sporis (etiam ex cystocarpio juveni) colorem atriorem parti medianae zonae medianalis communicantibus.*—EDITOR, BOTANICAL GAZETTE.

throughout the winter, so that all stages of the plant have been observed and carefully studied. The broad antheridial band, the rather prominent antheridial zone, and the constricted procarp zone resemble in a rather striking manner *L. nodosa*, but the *Chantransia* form is very different, as can be seen by comparing SIRODOT'S fig. 75 and ATKINSON'S figs. 23-26 of *L. australis*.

The material collected at Columbia, South Carolina, clearly belongs to this species. Other material from Maryland, West Virginia, South Carolina, Georgia, and Mississippi is assigned here partly because of the general agreement of the mature sexual shoots, and partly because of the regional distribution. The color of the sexual shoots of this species varies considerably under different conditions. When fresh, before they are very old, the shoots are green or olive-green in color; after standing in quiet water for a time or in the laboratory, however, the violet color often appears and the water in which they are left for some time is stained somewhat violet. Specimens which are dried often undergo slight disintegration changes in which this violet color appears, so that dried material of young shoots, or of old shoots in mass, is often more or less violet in color, together with the dark or blackish color which often appears when the plants dry. In this condition, when moistened in water, individual filaments under the lens often show an olive-green color with a tinge of violet (or in some sexual shoots the violet color is marked), while to the unaided eye there is a distinct violet tinge. Specimens from Rocky Creek near Chester, South Carolina, in the young stage show, strikingly, characters of typical *L. australis*, in which many of the more slender specimens show only a slight undulation, thus resembling *L. grandis*; but the larger and more robust ones have a strong undulating outline, corresponding with the undulating antheridial and procarp zones. These different forms can never be assigned satisfactorily to the proper species until the young stages are collected, including well developed *Chantransia* forms and the sexual shoots at the time of fertilization.

Georgia: on submerged limestone rocks in Burnt Mill Creek, alt. 840 ft., Walker Co., *Percy Wilson*, Aug 3, 1900 (N.Y.); (C).

Maryland: in stream, Garrett Co., *J. D. Smith*, July, 1878 (U.S.).

Mississippi: on rocks in running water, Meridian, *S. M. Tracy*, June 2, 1897 (N. Y.); (C).

North Carolina: in rapids, Morgan's Creek, Chapel Hill, *G. F. Atkinson*, winter and spring of 1886-1888, Phyc. Bor. Am. no. 38 (C).

South Carolina: on large stones in Broad River, Columbia, *G. F. Atkinson*, March and June, 1889 (C); Stroud's Ford, Rocky Creek, 4 miles east of Chester, *H. A. Green*, Nov. 23, 1895; Tilden's Am. Algae no. 108, May, 1890 (C).

West Virginia: Blackwater Falls, *W. C. Sturgis*, July 17, 1889 (Calif.).

LEMANEA CATENATA Kütz. Phyc. Germ. 261. 1845; Sp. Alg. 528. 1849; Tab. Phyc. 7:34. (pl. 87. fig. 1.) 1857.

The specimens examined from North America are up to 10 cm. long, olive-green when young and fresh, becoming darker in age, and on drying sometimes of a blackish or blackish-violet color; many shoots, however, still show the olive-green color. Antheridial band narrow, antheridial zone more or less angular, especially when young or medium-grown, but not papillate or tubercular.

The specimens collected at Corkscrew Falls, California, appear to represent typical specimens of this species, although they are small and do not show the strong "*catenate*" character of large examples. They compare well with the smaller authentic specimens in SIRODOT's *Exsiccata*.

California: Corkscrew Falls, San Bernardino Co., *S. B. Parish*, June 22, 1895, no. 94478 (Calif.); in Kern River, *N. L. Gardner*, Nov., 1916, no. 3507 (Calif.); on rocks, Crane Creek Falls, above El Portal, *W. A. Setchell*, June, 1916, no. 6509 (Calif.).

LEMANEA CATENATA Kütz. forma *CAPILLACEA* Sirodot. Ann. Sci. Nat. Bot. Ser. V. 16:80. 1872.

Sexual shoots dark olive-green in age with a faint violet tinge, blackening when dried, slender, straight or slightly curved, not rigid, up to 12 cm. long; tapering gradually at base; antheridial region often somewhat angular and prominent. Antheridial bands not very distant, rather narrow, irregular toward base, often interrupted, tissue beneath the antheridia in age often hypertrophied and then sometimes irregular; procarp zone nearly or quite cylindrical; procarps in middle of procarp zone, spores at maturity often filling procarp zone. In these specimens the spores are not mature and do not darken the sexual shoots. The *Chantransia* stage had disap-

peared, and therefore is not available for comparison with SIRODOT'S description.

California: West Canyon, Palm Valley, Riverside Co., Eastern (Desert) base San Jacinto Mt., plants of Southern California, *S. B. Parish*, Apr., 1896, no. 4055 (Calif.); (C).

LEMANEA GRANDIS (Wolle) Atkinson *Ann Botany* 4:219. figs. 46, 50. 1890.

The sexual shoots of type material received from Wolle, when moistened in water are of a sordid olive-green color; nearly straight to more or less strongly curved and measure 4-6 cm. \times 0.5-0.8 mm. They are mostly cylindrical but taper toward either end, more gradually so toward the base, rather blunt at the apex, where they have been probably broken and then healed. A few of them show a slight undulation of outline, corresponding in these cases to the slightly broader diameter of the antheridial zone and the slightly but gradually constricted procarp zone.

WOLLE says that "the specimens were growing on stones in rather sluggish water in the river at Bethlehem, Pa." Probably in deeper water where there is a stronger current the sexual shoots would be somewhat stouter.

Specimens collected in a stream at Falkland, Delaware, by *A. Commons*, in 1886, have been assigned to this species. The sexual shoots are somewhat stouter, rather strongly curved, and the greater freedom of the shoots from extraneous matter seems to indicate that they were growing in deeper water where the current was probably stronger than in the case of the specimens collected by *Wolle* at Bethlehem. The sexual shoots of these specimens, moistened in water, show under the hand lens a dark green color. To the unaided eye, or in mass, they are darker in color, with a suggestion of a violet tinge. Some younger specimens, when moistened in water, show this violet tinge to the unaided eye, although under the lens the color is more a dark, dull olive-green. These shoots are mostly cylindrical, but a large proportion show slight undulation in consequence of the alternation of the broad antheridial zone with the slightly constricted procarp zone.

Delaware: in river, Falkland, *A. Commons*, 1886 (F); (C). Pennsylvania: on stones in shallow, sluggish river water, Bethlehem,

Frances Wolle. 1876-1878, Rabenhorst's Alg. Europ. no. 2538 (N.Y.).

Lemanea mexicana Kütz. Tab. Phyc. 7:34. pl. 86, fig. 2. 1857.

Sexual shoots in tufts, simple or sometimes branched, the branching probably abnormal, resulting from injury or hypertrophy, with regeneration of several to numerous sexual shoots in the region of injury; at and soon after fertilization olive in color, olive-brown when dry, specimens examined stout, rather strongly curved, 4-6 cm. long by 1-1.25 mm. in diameter; wall rather thick, firm and subcartilaginous, at time of fertilization and soon after cylindrical or nearly so, antheridial region only slightly stouter, procarp region cylindrical or very slightly and gradually constricted; antheridial band broad and regular, of a slightly darker tint than the wall of the shoot; often presenting several blunt papillae, or sometimes with papillae very numerous on antheridial zone and elsewhere, probably due to some epiphyte; procarps in the middle of the procarp zone; spores in this material few, young.

Mexico: in stream, Orizaba, *Botten*, Herb. Sullivant.

These specimens were made available for examination through the kindness of the late Dr. W. G. FARLOW who also wrote concerning them as follows: "There is no date on *Botten's* specimen. *Botten* was sent by the London Hort. Society to Mexico in 1850 to collect. Somewhat later he settled at Orizaba where he collected independently. He did not die until 1885, but the specimens of *Lemanea* came from Herb. Sullivant and, as S. died in 1873, the chances are that the *Lemanea* was collected between 1850-70." It is possible that KÜTZING's species was based on material collected by *Botten* for the London Hort. Soc. The appearance of the sexual shoot shown in KÜTZING's fig. C^r resembles the specimens from Orizaba in the warty character, some of these warts very probably being due to an epiphyte.

Lemanea pleocarpa Atkinson, sp. n.

Sexual shoots in dense tufts, rather stout, 8-20 cm. long, purplish brown when dry, when soaked in water showing a slight purplish tinge but the color is pale, often with portions olive-green, rather abruptly tapering at base, which is more or less curved, antheridial zones not prominent, antheridial band narrow and interrupted below, over the middle and terminal portions broader and more regu-

lar; procarp zone only slightly tapering from antheridial zone except near base in very stout plants where it is more strongly constricted, procarps numerous extending throughout the procarp zone even into the antheridial zone. Carpospores large but rather young, many of them quite young in the specimens examined, *Chantransia* form not seen.⁵

In the more general distribution of the procarps this species resembles *Lemanea fluviatilis* but differs in the subgeneric characters. Although in both species there is a wide distribution of the procarps, in this species they are more abundant in the middle of the procarp zone, while in *L. fluviatilis* they are more abundant in the antheridial zone.

The specimens from Kentucky are younger and more vigorous plants. When pressed upon paper the "torulose" appearance is more striking and the antheridial zones show more prominently. The specimens were remarkably well preserved. In the collections from both localities the plants have a purplish-brown tinge when dry, the older ones from Beaver Creek blackish, although the purplish-brown tinge when soaked in water shows also in the younger plants from Kentucky, under the microscope. There are many places on the filaments which also show a greenish color. Whether the plants are entirely or partly green when fresh is not known.

This is a very interesting species. It is the only one in this section of the genus, which has come under observation, in which procarps and cystocarps are present so near to, and in, the antheridial zone. It is interesting also from a historical point of view, the specimens collected by *Dr. Peter* (1834) and *Dr. Short* (1842) being the earliest collections for North America. The specimens from Kentucky collected by *Dr. C. W. Short* are cited as the type of *Lemanea pleocarpa*, with those collected by *Dr. Peter* as a cotype. The latter specimens,

⁵ In accordance with the requirements of the International Rules of Plant Nomenclature, the description is here Latinized: Stirpes sexuales dense torulosae, subrobustae, 8-20 cm. longae, exsiccatae purpureo-brunneae, maceratae vix subpurpureae sed pallidae, saepe nonnullis partibus olivaceo-virides, subabrupte attenuatae ad basim plus minusve arcuatam; zonis antheridialibus non valde perspicuis, vitta antheridiali angusta atque inferne interrupte . . . ; zona procarporum e zona antheridiali tantum paululo attenuata (nisi prope basim pro plantis robustissimis valdius constricta), procarpiis numerosis per omnes partes suae zonae et etiam in zonam antheridialem sparsis. Carposporae magnae sed subjuvenes (pro speciminibus visis multae juvenissimae); *Chantransia* forma non visa.—EDITOR, BOTANICAL GAZETTE.

which were examined, were from the herbarium of Judge DOUGLAS HORTON and are now in the herbarium of the University of Michigan.

HARVEY (Nereis Bor. Am. 67. 1858) reports specimens collected by Short in rivers and streams of Kentucky as *L. torulosa*, and makes the interesting comment that the "Globose masses of fructification are attached to the inner surface of the tubular frond, either at the nodes or between them without apparent order."

Kentucky: on rocks in brook near Lexington, Robert Peter, March, 1834 (M); on rocks and stones, in rivers and small streams, C. W. Short, March, 1842 (C).

Virginia: in Little Falls, Beaver Creek, near Marion, Anna M. Vail and Elizabeth G. Britton, June 21 and 26, 1892 (C).

LEMANEA TORULOSA Sirodot. Ann. Sci. Nat. Bot. Ser. V. 16:82. pl. 1, fig. 7; pl. 6, figs. 41, 42; pl. 8, fig. 77. 1872.

In this species the sexual shoots are nearly even throughout, the procarp zones being only slightly and gradually, if at all, narrowed, especially at maturity. When fresh the color is olive with a greenish tinge. All the specimens referred here are small. No material of the *Chantransia* form was examined, but the material from a mountain rivulet, Pa. (Herb. Wollé) appears to be typical so far as can be judged from mature sexual shoots alone. That from Wissahickon Creek is badly parasitized by a species of *Onchybursa*. The collections from Virginia, formerly referred doubtfully to *L. nodosa* Kütz. (Atkinson, Ann. Botany 4:218, 1890), should be referred here pending a study of good material of both stages.

Pennsylvania: on stones in mountain rivulet, at Narrows, F. Wollé, July 1, 1874 (appearing to be good *L. torulosa* although small; Herb. Wollé); on rocks in Wissahickon Creek, Oct., 1854 (F); (C).

Virginia: on rocks in rapids of James River, Richmond, G. F. Atkinson, June, 1888 (C); in rapids of small stream near Fredericksburg, G. F. Atkinson, June, 1888 (C).

SECTION SACHERIA

Lemanea (Sacheria) fluviatilis (Linn.) Ag. (as *Lemania*), Dispos. Algar. Sv [Sv.] Vet.-Acad. Handl. 1814: 40; 45. pl. 2, fig. 2. 1814; Spec. Alg. 2:4. 1824-1828.

Oregon: in brook (F). These are typical specimens but collector and date unknown.

BAILEY (Am. Jour. Sci. Ser. II. 3:185. 1846) reports *L. fluxiatilis* Ag. from Cascade, West Point; Mountain Run, Culpepper Co., and falls in the Rappahannock River above Fredericksburg, Va. The collection from the last named locality proved to be *Tuomeya fluxiatilis* Harv. The specimens from the two other localities given by BAILEY do not appear to be preserved in his collection, and it is not possible to say what species they represent; but they probably do not belong to *L. fluxiatilis* Ag.

Lemanea (*Sacheria*) *fucina* Bory. Ann. Mus. Nat. Hist. 12:185. pl. 23, fig. 3. 1808.

This is a highly variable species in the form and branching of the sexual shoots, as previously pointed out (ATKINSON, 1890: pp. 224, 225). The variations relate to the form of the procarp zone, the prominence of the antheridial nodules, and the branching. Four of these forms were recognized as species by SIRODOT, but in this country the forms so grade one into another that it seems preferable to treat them merely as varieties.

Var. *β. mamillosa* Atkinson. Ann. Botany 4:225. figs. 1-5, 8, 9, 11-18, 55, 56, 58. 1890.

North Carolina: Bolan's Creek and Morgan's Creek, near Chapel Hill, *Geo. F. Atkinson*, winter and spring, 1886-1888. Phyc. Bor. Am. no. 37 (C).

Var. *γ. subtilis* Atkinson. Ann. Botany 4:225. fig. 54. 1890. *Sacheria rigida* Sirodot. Dame and Collins, Flora of Middlesex County, Mass. 153. 1888.

Massachusetts: on rocks in very rapid water, The Cascade, Middlesex Falls, Melrose, *F. S. Collins*, May 4, 1879 and April-June, 1883; Apr. 14, 1889 and April 15, 1894, Phyc. Bor. Am. no. 36a and b (C); in stream, Arnold Arboretum, Forest Hills, *B. M. Davis*, May 3, 1895; in streams, Malden, *F. S. Collins*.

Var. *δ. rigida* Atkinson. Ann. Botany 4:225. figs. 6, 7, 10. 1890. *Lemanea borealis* Atkinson, *Torrey* 4:26. 1904.

This is the most common variety of the species and is widely distributed in North America. There is considerable variation in the sexual shoots, especially in size. Much of the material collected in the summer or early autumn is badly water-worn. The most important variation is shown in material from Oregon, which varies toward *L. fluxiatilis* in the presence of procarps and spore groups

near the middle of the procarp zone, but not so evenly and regularly distributed, and lacks the color of the sexual shoots of typical *L. fluviatilis*. Material from Nova Scotia, especially that from Pirates Cove, and from Newfoundland (Bay of Islands) presents similar variations. That from Bay of Islands, N. F., was made the type of a new species. Some material from this section, however, cannot be separated from the more typical *rigida*, and an examination of SIRODOT'S *Exsiccata* reveals one collection of his *Sacheria rigida* in which the procarps and spore groups, here and there, approach the middle of the procarp zone. The irregularity of this distribution in SIRODOT'S and our material appears to be due to some unfavorable condition of development rather than to genetic differentiation. It is possible that when material from these widely separated localities can be studied carefully in different stages of development, specifically distinct genetic forms may be found; but for the present it does not seem reasonable to attempt a differentiation.

California: in stream at Little Bear Valley, San Bernardino Mts., *S. B. Parish*, Aug., 1884 (F); (C); on rocks in waterfall, Sacramento River, Limo, Shasta Co., *M. A. Howe*, Aug. 10, 1894 (N.Y.); (C).

Colorado: in cold swift rivers, alt. 7000-9000 ft., near Hot Sulphur Springs, Grand Co., *E. Bethel*. No date given, but the envelope containing plants bears the stamp, "Dec. 7, 1911."

Connecticut: Island Brook Bridgeport, *Isaac Holden*, various collections June to Dec., 1888, 1889; *W. A. Setchell*, 1887 (Calif.); (C); Trading Cove Brook, Norwich, *W. A. Setchell*, June 29, 1890 (Calif.); Stony Brook, Montville, *W. A. Setchell*, June 30, 1890 (Calif.); (C); Mill River, Bridgeport, *Isaac Holden*, July 5, 1891; Great Falls, Housatonic River, *Isaac Holden*, June 27, 1891, Phyc. Bor. Am. no. 34c (C); in Ironworks Brook, North Guilford, *D. C. Eaton*, May, 1887 (Y); (C); on stones in swift-running water, Sargents River, Woodbridge, *O. D. Allen*, June 29, 1880 (C); on stones in Hammonasset River, Killingworth, *F. W. Hall*, July, 1874 (F); (C); in stream, Franklin, *W. A. Setchell*, July 2, 1888 (Calif.); (C); Westrock Cascade, New Haven, *D. C. Eaton*.

Maine: on rocks in swift water, Sunk Haze Stream, Greenfield, *E. D. Merrill*, Sept., 1897; on ledge in bed of large brook, Camden, *Alice L. Crockett*, July 10, 1904 (N. Y.); (C); on stones in little

brook near Round Pond, *F. S. Collins*, July 14, 1901 (C); outlet of Echo Lake, Mt. Desert Island, *Isaac Holden*, Aug 11, 1889

Maryland: in stream, Garrett Co., *J. D. Smith*, July, 1878 (U S.).

Massachusetts: Bussey Brook, Jamaica Plain, *E. Faxon*, May 3, 1883 (F); (C); waterfall, Waverly, *A. B. Scymour*, 1913 (F); on rocks in bed of rapid stream, Chester, *Dr. E. Emmons* (N.Y.); in rapids of stream below mill dam, Milltown south of Boston, *G. F. Atkinson*, July, 1888 (C); on mill dam in swift current, Arlington, *W. A. Setchell*, June 11, 1890, Dec. 21, 1890, Phyc. Bor. Am no. 34a, b (C).

Minnesota: Lester River, Duluth, *C. Bullard*, Aug., 1902 (F).

Mississippi: on rocks in running water, Meridian, *S. M. Tracy*, June 2, 1897 (N.Y.).

New Hampshire: in Mill Brook, Shelbourne, *W. G. Farlow*, Aug., 1882 (F); (N Y.); (C).

New York: rapids in Cascadilla Gorge, Cornell Univ. campus, *G. F. Atkinson*, winter and spring, 1885 (C); Triphammer Falls and rapids in Fall Creek, Cornell University campus, Ithaca, N.Y., *W. R. Dudley*, June, 1885, *G. F. Atkinson*, July, 1886; on rocks Taughannock Falls, *W. R. Dudley*, Aug 1, 1885 (C); lower Taughannock Falls, *W. C. Muenscher*, Apr., 1921⁶ (C); on trap rock in brook by mill, Johnson Pond Road, Adirondack Mts, *E. G. and N. L. Britton*, Sept 6, 1900 (N.Y.); (C); on rocks, Twin Glen, Ithaca, *W. C. Muenscher*, May 18 and Dec. 7, 1928 (C); Remington Brook, Ithaca, *W. C. Muenscher*, Dec., 1928 (C); on rocks in rapids of St. Regis River, Brasher Falls, *W. C. Muenscher*, Sept. 6, 1930 (C); in rapids below chasm, Chateaugay River, *W. C. Muenscher*, Sept, 1930 (C); Point Rock Creek, Adirondack Mts., *R. Laubengayer*, July, 1928 (C); on stones in rapids of the Bronx River near the Lorillard Mansion, Bronx Park, N.Y. City, *F. T. McLean*, May 9, 1902 (N.Y.); (C); Bashbish Falls, *R. S. Williams*, Aug. 5, 1900 (N. Y.); (C); on rocks, Conglomerate Ravine, Ellenville, *E. G. Knight* and *N. L. Britton*, Aug. 25, 1883 (N.Y.); (C); on stones in swift water, Pond Brook 2 miles east of Fort Ann, Washington Co., *S. H. Burnham*, June 3, 1914 (C).

⁶ A few specimens not seen by ATKINSON, those added to the herbarium of Cornell University since 1918, have been included in these notes on distribution.

North Carolina: Oates Falls, *R. Thaxter*, 1896.

Ohio: in stream, Painesville, *Dr. Beardsley* (N.Y.), (C).

Oregon: on stones in creek near Hood's Ranch, National Forest, *F. V. Coville*, June 25, 1907 (U.S.); on rocks in Power's Creek, Silverton, *A. S. Foster*, no. 1228 (N.Y.); in stream, Forest Grove, *A. R. Sweetser*, May, 1903 (F); Oregon, *Elihu Hall*, 1871 (F).

Pennsylvania: Buttermilk Falls, July 4, 1898 (N.Y.); Stony Creek, *F. Wolle*, July, 1875 (C); in rapid water, Bark Hill, *F. Wolle*, 1874, 1877; in bed of brook, Saw Kill Falls, Pike Co., *G. V. Nash*, July 20, 1909 (N.Y.); (C).

Vermont: Otter Creek Falls, Vergennes, *C. P. Mott*, June 29, 1894; Little Otter Creek, Ferrisburg, *E. Faxon*, July 22 and 28, 1884 (F); (C).

Virginia: in rapids, Potomac River, Harper's Ferry, *E. S. Burgess*, June 5, 1888 (C).

West Virginia: Blackwater Falls, Cheat River, *J. D. Smith*, July 5, 1878 (U.S.).

Canada: on rocks in river at Bellville, *J. Macoun* (N.Y.); (C); on rocks in stream, Owen Sound, Ontario, *J. Macoun*, no. 262.

New Brunswick: in Nepsiquit River, *J. B. Fowler* (F).

Newfoundland: on rocks in waterfall, Bay of Islands, *C. D. Howe* and *W. F. Lang*, Aug. 9 and 10, 1901 (N.Y.).

Nova Scotia: in stream, Pirates Cove, *J. Macoun* (F).

British Columbia: in little waterfall, head of Departure Bay, Vancouver Island, *Mary J. Macoun*; not typical.

Var. *e. viviana* Atkinson, *Ann. Botany* 4:226 fig. 53. 1890. (*Sacheria rigida* var. *viviana* Sirodot *Ann. Sci. Nat. Bot. Ser. V.* 16:73. fig. 87. 1872.)

Connecticut: Island Brook, Bridgeport, *Isaac Holden*, Dec., 1888 to June, 1889, *Phyc. Bor. Am.* no. 35 (Calif.); (C); Stillman's Brook, Bridgeport, *Isaac Holden*, May, 1889 (C).

Massachusetts: Bussey Brook, Jamaica Plains, *E. Faxon*, May 9, 1883 (F); (C); Cascades, Waverly, *B. M. Davis*, May 11, 1894.

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[Accepted for publication April 3, 1931]

THE SUSPENSOR OF SCIADOPITYS

JOHN T. BUCHHOLZ

(WITH NINETEEN FIGURES)

A peculiar suspensor which has seemed unique and anomalous was described for *Sciadopitys* by ARNOLDI (1). This observation has not been confirmed, and our present knowledge of the embryogeny remains fragmentary. The suspensor is composed of two sections which have a group of small cells between the elongated portions. ARNOLDI described also a group of proliferating cells above the suspensor, and likened this feature to a protocorm. Doubtless the object which he observed was a group of rosette cells or embryos. LAWSON (17), who observed some of the early stages of the embryo, did not describe the rosette, nor did his observations extend into the slightly later stages, which include the formation of the interrupted suspensor figured by ARNOLDI. LAWSON concludes: "on the whole the embryo of *Sciadopitys* is rather unique; it does not bear a close resemblance to either Abietineae, Cupressineae, or Taxaceae." COULTER and CHAMBERLAIN (10) regarded *Sciadopitys* as one of the Taxodineae. The writer has observed a very similar suspensor in *Biota* (6), one of the Cupressineae often erroneously included with *Thuja*, and this discovery has stimulated the present investigation into the embryogeny of *Sciadopitys*. The general situation in *Sciadopitys* was inferred and given in a previous publication (4), in an attempt to harmonize the facts as stated and illustrated by ARNOLDI and LAWSON, but this summary was not then based upon an independent personal investigation.

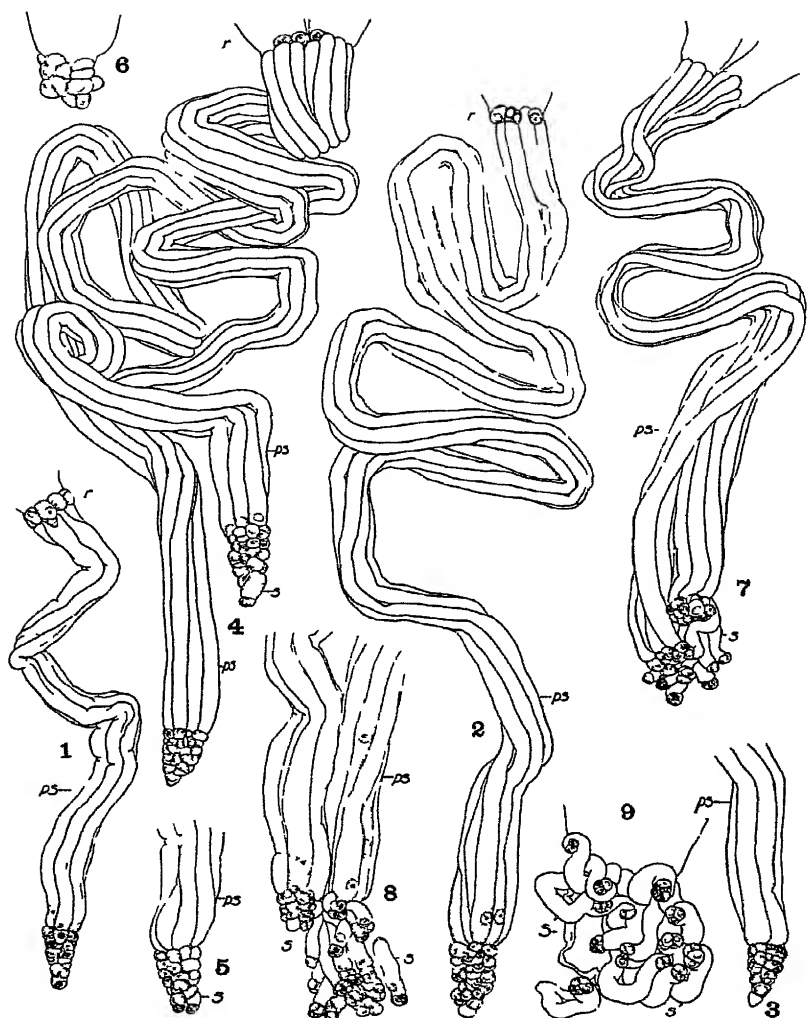
Material and methods

The material used in this investigation was obtained from three sources. Through the kindness of the Director of the Kew Botanical Gardens, Dr. A. W. HILL, arrangements were made for a number of collections of ovules which were killed there in formalin-alcohol at weekly intervals during the summer of 1926. A few cones of *Sciadopitys* were collected also from one of the trees growing on the grounds

of the Country Life Press, Garden City, New York, in the summer of 1926, but the embryos of these were found to be too far advanced to include the early embryogeny; they furnished the examples figured in the oldest stages represented. In the summer of 1927, a single cone was obtained through Kelsey's Nursery from plantings in the region around Salem, Massachusetts. This cone was large and well developed, and contained many embryos in the stages shown in most of the early figures described here. The earliest stages (figs. 1-5) were dissected out on July 12 and 14, and some later stages (figs. 7-9) on July 17, from the ovules of a part of the same cone, which was kept packed in damp moss in the laboratory.

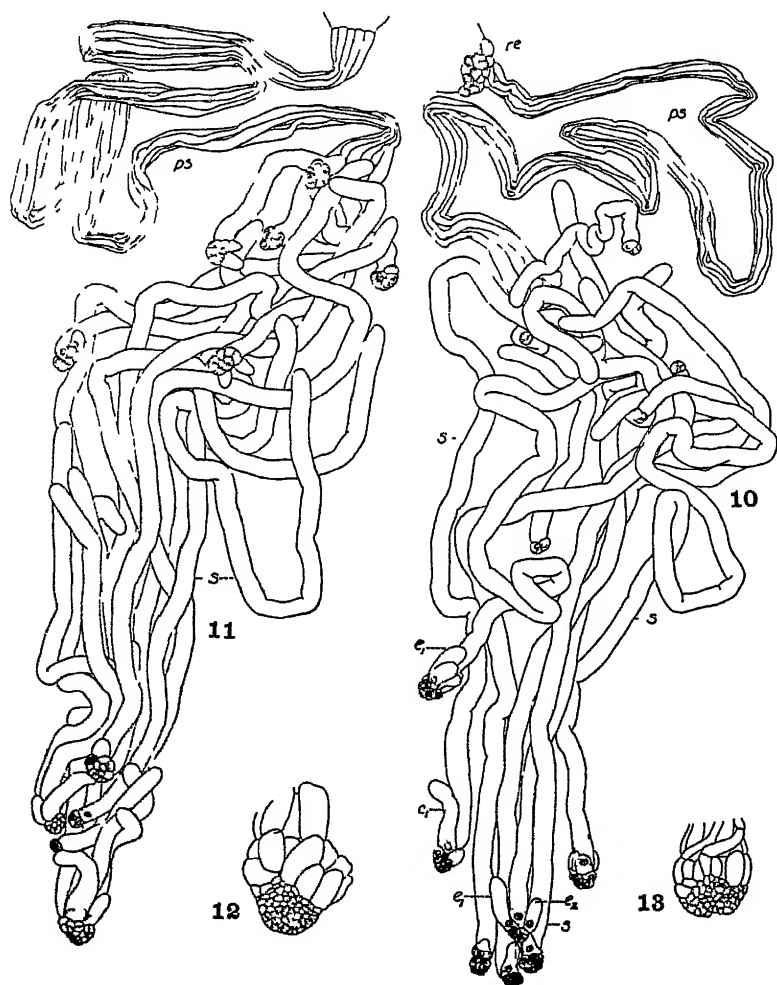
The Kew material, which was collected in June, 1926, contained unfertilized eggs; that of July 2 contained a few embryos of the stages shown in figs. 2 and 3; that of July 9 contained embryos of the stages shown in figs. 10 and 11 and older; and that of July 20 contained embryos of the stages shown in figs. 12-14. This material was killed and preserved for many months before dissection. It came from cones collected at different times and possibly from different trees, which indicates that the stages shown in figs. 7-9 were largely missed, because this stage is passed through rapidly; and the stages of the different collections did not correspond closely. In part, the difficulty was due to unsatisfactory dissections of these particular stages from the ovules of preserved material, with the loss of some of the parts which belong to the same embryo system and should hang together. Aside from the difficulties in the dissection of preserved material, there were so many loose endosperm cells adhering to the suspensors that they were difficult to interpret. In the study of conifer embryos by dissection, it has always been found advantageous to have the living material, although preserved material is sometimes useful when properly killed and fixed. In general, the same conditions were found in the Kew material as in the Salem cone in slightly older stages. The stages represented in figs. 4, 7, and 8 are passed through so rapidly, that in order to make certain to obtain this stage it would probably be necessary to make collections during 3- or 4-day intervals.

The embryos were stained in Delafield's haematoxylin and mounted in diaphane, following the general method described elsewhere (6). The drawings were made with a camera lucida, and are



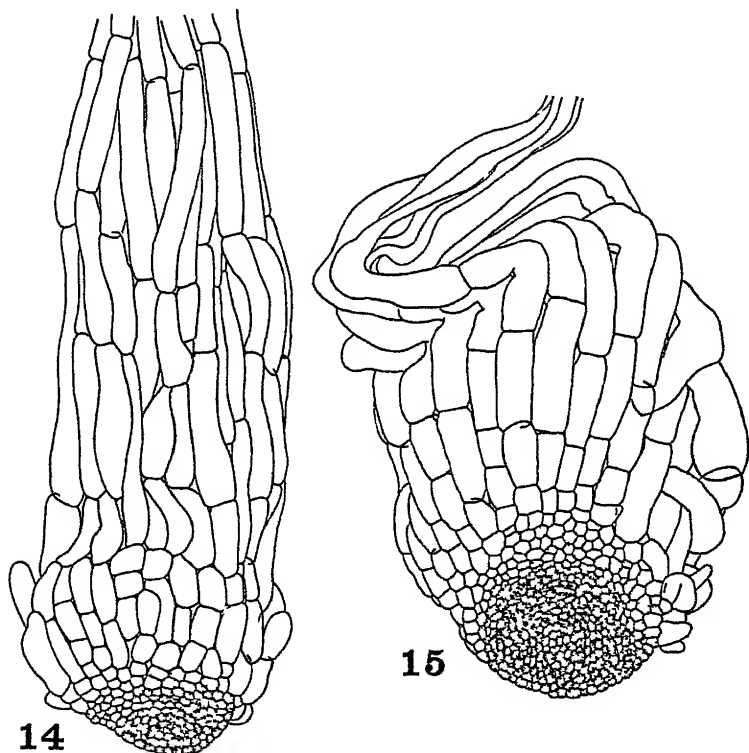
FIGS 1-9—Fig 1, embryo system in earliest observed stage in embryogeny: *r*, group of rosette cells, *ps*, prosuspensor. Fig 2, embryo system after prosuspensor is fully elongated, with aborted cell below at tip and another at side (only 3 rosette cells found above prosuspensor, central one of which has divided). Fig 3, lower end of another similar embryo system. Fig 4, embryo complex composed of two adjacent embryo systems with a few scattered rosette cells above, embryo system at left shows no aborted cells, while in the one at right a dead cap cell has been pushed aside by elongation of a primary suspensor (*s*) of a 2-celled embryo unit. Fig 5, lower end of another embryo system in same stage. Fig 6, embryo system still attached to archegonium in absence of prosuspensor, several embryos have reached 2-celled stage. Fig 7, complex of two adjacent embryo systems in which many embryo units are producing primary suspensors while others still remain without elongated primary suspensors, giving appearance of suspensor system in two sections with small cells between. Fig 8, similar stage in which embryo system at left remains in stage of fig. 2, while the one at right is slightly older than systems of fig. 7 and contains many 3- and 4-celled embryos, on elongating primary suspensors. Fig 9, embryo system similar to that of fig. 6, with primary suspensors of individual embryo units elongating; $\times 50$.

accurately reproduced in respect to the embryonic structures; but even in the material dissected in living condition, the suspensors were usually covered with some scattered loose endosperm cells



FIGS. 10-13.—Fig. 10, embryo system after primary suspensors (*s*) have become very long and have pushed the collapsed prosuspensor up toward micropyle; rosette embryos (*re*) ready to form primary suspensors; embryonal tubes (*e*₁ *e*₂ etc.) beginning to elongate from many embryos. Fig. 11, similar condition in more advanced stage, for embryo system which has no rosette embryos. Figs. 12, 13, successive stages in development of individual embryos after secondary suspensors have become multicellular; $\times 50$.

omitted from the drawings. These could easily be recognized by their position and shape, their starch content, and their multinuclear condition. All drawings are surface views of dissections, and show the structures observed in the highest planes of focus.



FIGS 14, 15.—Successive stages in development of individual embryos after secondary suspensors have become multicellular; $\times 75$.

Some of the older stages of the embryo have been imbedded for sections. These may be described in greater detail and included in a comparative study, now in progress, of the later stages of various conifer embryos. It is intended here to describe the development of the suspensor and early embryo, including the peculiar conditions of cleavage polyembryony found in this genus. This includes figures of the suspensors similar to those which ARNOLDI described, as well as later stages.

Suspensor formation

Fig 1 represents the earliest normal stage which could be found in this material. At this stage the characteristic twisting and coiling of suspensor cells found in conifers has begun. This is the beginning of an embryo system which includes all the embryonic parts derived from a single archegonium. The four or five archegonia are terminal, are somewhat deeply imbedded, and are not grouped into an archegonial complex as in the *Taxodineae* and *Cupressineae*, although they seem to lie close together. ARNOLDI (1) found an occasional lateral archegonium, while LAWSON (17) did not mention lateral archegonia.

It is evident that the proembryo which was not observed must consist of at least three regions: the lower which includes the group of embryonic cells shown below in fig. 1, the suspensor cells which elongate, and sometimes a group of rosette cells above these. There is probably an additional tier of free nuclei above the rosette, as in some other conifers, which would give a proembryo formed of four tiers. Fig 1 is already advanced to a stage in which traces of such a group of nuclei would have disappeared.

Not all the embryo systems have a rosette group, as indicated in the rosette region of figs. 7 and 11; in fact, these cells are more commonly absent or reduced to only a few cells, as shown in fig. 4. When the rosette is absent, it is probably the uppermost layer of cells which elongates as a group of suspensor cells. This condition would presuppose fewer tiers in the proembryo, or that more of the embryonic cells were included in the terminal group below. In order to obtain a statistical estimate of the prevalence of rosette cells, 100 embryo systems were examined. Of these, 15 had conspicuous rosettes of many cells, similar to the rosette in figs. 1 and 10; 6 others had similar rosette groups which had nearly disintegrated; 47 had only 1-3 or 4 rosette cells, as in figs. 2 and 4; 29 had no rosette cells at all, as shown in figs. 7 and 11; and 3 systems were found without the large elongated prosuspensor, as shown in figs. 6 and 9.

Elsewhere (6) the writer has found it necessary to allude to the group of suspensor cells (*ps*) which elongate from the proembryo as a "prosuspensor," and it is appropriate to use this terminology here. Likewise it may be less confusing to state at the outset that the

embryo system of fig. 1 is in reality a group of many embryos, mostly in the 1-celled stage. These cells, therefore, are embryo initials, some of which are dividing or have already divided into 2-celled embryos at this stage. The number of lower embryo initials is 12 to 18 or more; the number of cells in the prosuspensor (*ps*) is usually 7 to 9; the rosette cells (*r*) 0 to 9 or more; and the number of free nuclei above the suspensor, if there are any, is unknown.

A number of the embryo initials are sometimes found undergoing disintegration as the prosuspensor becomes elongated. In figs. 1 and 5 all of the embryo initials which are visible appear to be living and active, while figs. 2 and 3 each show a terminal embryo initial which has become transparent and empty. This empty cell is in the position of a cap cell, resembling the cap cell of *Podocarpus spicatus* (6, 21) and *Cephalotaxus* (5, 9, 15, 22). Fig. 4, which is made up of two embryo systems, appears to be without such a cap cell in the embryo system at the left, while in the system at the right a sterile cell is still visible near the tip. As stated, other cells anywhere in this terminal group may die or become crushed as the prosuspensor is elongated. It is evident, therefore, that through abortion of embryonic cells, the number of embryo-initial groups which are found in later stages may vary considerably.

That the prosuspensor may become exceedingly long is indicated in the figures of the subsequent stages (figs. 2, 4). It becomes greatly coiled and twisted as it pushes the terminal group of embryonic cells deep into the prothallium. No doubt this mechanical action is responsible for the crushing of some of the embryonic cells. A digestive enzyme is present which corrodes the surrounding prothallial tissue, and soon creates a funnel-shaped cavity, flattened slightly and extending downward from the archegonia. This cavity in which the embryos lie is not always clearly bounded in these early stages, for the embryos are frequently found tightly imbedded in the spongy cells, and are not easily removed by dissection; when removed many of the loosely scattered endosperm cells of the gametophyte may remain attached to the surface of the prosuspensors and embryonic cells.

The nuclei of the early embryonic cells (figs. 1-5) are large, and are bounded by a narrow margin of dense cytoplasm, free from

vacuoles. From 2-celled embryo initials individual primary suspensors (*s*) may begin to elongate before the prosuspensors have attained their maximum length. When this elongation begins, each embryo unit may be 2-celled, but is frequently 3- or 4-celled. The system shown at the right in fig. 4 has a terminal embryo, with the upper one of its two cells (*s*) beginning to elongate to form a primary suspensor. Fig. 5 shows a slightly earlier stage in two similarly situated 2-celled embryos, while in figs. 7 and 8 many of the embryos have become 3- or 4-celled. It will be seen that the small embryos are variously oriented and begin to elongate in all directions, but in general they tend to grow downward.

This stage finds the group of embryo initials thrust well down into the prothallium. For a brief period, while the embryo initials have not all produced primary suspensors, the entire suspensor system gives the appearance of a suspensor in two sections with the suspensorless embryonic cells between. These stages are illustrated by figs. 7 and 8, where in each case two embryo systems are shown side by side. It is clear, therefore, that the "small cells between two sections of suspensor" represent embryo initials situated between the prosuspensor and the primary suspensors of slightly more advanced embryonic units.

During the early stages, the individual cells of the prosuspensor have a large single nucleus, usually found in the lower end near the embryo initials. When the prosuspensor has become fully elongated and its cells are coiled and twisted, the nuclei disappear and the prosuspensor soon begins to collapse (fig. 7). Figs. 10 and 11 show the collapsed prosuspensors coiled back in the archegonial region, where they have been crowded into this position by the primary suspensors, as these have elongated upward from individual embryo units in the process of pushing the latter farther and farther downward. When the primary suspensors have become very long, as in fig. 10, some of the other adjacent cells of the embryo begin to elongate, initiating the formation of embryonal tubes which make up the secondary suspensor. As the number of these increases, the secondary suspensor becomes more and more massive (figs. 10-15).

Fig. 6 shows the lower portion of an archegonium in which a prosuspensor did not elongate. The embryonic cells are mostly ad-

vanced to the 2-celled stage, but are still attached to the base of the archegonium. Such conditions were rare, including about 3 per cent of the embryo systems. Fig. 9 represents an older stage similar to fig. 6, in which the embryos were slightly disarranged in mounting. Here are small embryos which, in the absence of a prosuspensor, are beginning to form primary suspensors at the base of an archegonium.

Apical cell growth

The embryos begin to develop by means of apical cell growth, but the apical cell is not always easily recognized, since orientation of the different embryo units varies considerably. The primary suspensor cell appears to be the first segment of the apical cell, which usually places its next wall nearly vertical to the first, and the following wall is inclined to both of the first two. It is probably an apical cell of three cutting faces. After twelve cells have been formed in each embryo, the apical cell with its last few segments may be identified when oriented to afford favorable side views. The embryos found in figs. 10 and 11 still appear to have apical cells, but in the stage shown in figs. 14 and 15 the embryos are advanced beyond the stage in which apical cells may be recognized. A close estimate of the number of cells in an embryo, when the apical cell disappears or is no longer recognizable, was not made, but it is safe to say that the number is much smaller than in *Pinus* (2), where as many as 500 cells have been estimated in some embryos before the apical cell disappears.

Polyembryony

Fig. 10 shows an embryo system in which twelve embryos may be counted on long primary suspensors below the collapsed prosuspensor. Those found in the terminal group far below are the largest. They are beginning to form embryonal tubes (e_1 , e_2) as secondary suspensor additions, which project backward and may diverge from various sides to form spurs. The number of these projecting spurs increases rapidly as the embryo enlarges, and fig. 11 illustrates how the terminal embryo is gaining the advantage over the others in pushing them back through the use of embryonal tubes. The suspensor becomes more massive by the addition of more and more embryonal tubes (figs. 12-15) which elongate from the

embryo. In fig. 14 the suspensor has gradually become more massive, while in fig. 15 the transition to a massive suspensor is more abrupt. The latter might give the outward appearance of ARNOLDI's fig. 27. I cannot confirm ARNOLDI's statement, however, that all of the product of a zygote may give rise to a single embryo. Each embryo system constantly splits up into many embryos. Obviously each of the small embryos which is beginning to elongate on an individual primary suspensor in figs. 7 and 8 continues independent development. For a time they all appear to be engaged in a struggle against the rest, until one of them gains an advantage and succeeds in pushing the others back toward the micropyle by means of an enlarging secondary suspensor.

Cleavage polyembryony of an extreme type is found in *Sciadopitys*. In spite of the loss of embryo initials through abortion in early stages, more embryos are produced per zygote than in any conifer which the writer has investigated. In *Biota* (6) sometimes as many or more embryos may be found in the entire embryo complex, but these represent a tangle of several embryo systems, which were actually contributed by several archegonia. The embryo system shown in fig. 10, which came from a single zygote, has twelve separate embryos, without considering the rosette. Many instances were found in which two or three systems were combined in stages older than figs. 7 and 8, but no attempt was made to illustrate them since they were too complex to be useful in this description.

ROSETTE EMBRYOS.—The rosette cells (*r*) shown in fig. 1 represent still other embryo initials which may begin to divide to form embryos. Fig. 2 shows only one of the three or four rosette cells divided. Fig. 1 shows three out of eight or nine rosette cells which have divided in beginning to form embryos, while the rosette of fig. 10 is in a slightly later stage. A few older stages have been found in which some of the rosette embryos were beginning to put out suspensors; also many more stages in which rosette cells were crushed and aborted. The fact that these cells divide (figs. 1, 10) and occasionally have short suspensors indicates that rosette cells, when present, are groups of embryo initials. If we estimate the number of potential embryos in the rosette from 0 to 9 and the embryo initials below the prosuspensor from 12 to 18, the possible

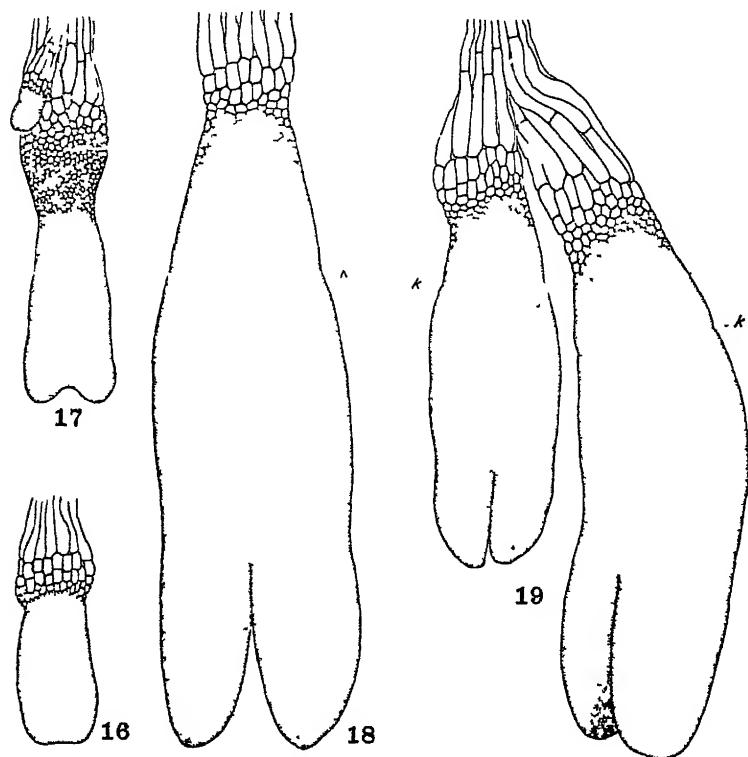
number of embryos from each zygote may reach 20-27 as a maximum, on the basis of the cleavage referable to the early zygote. This maximum estimate may be too high, for it is probable that when a maximum number of embryo initials is found below the prosuspensor, there are fewer of these included in the rosette.

BUDDING AND TWINNING OF EMBRYOS.—Following the stages of the embryos shown in fig. 11, they may become deeply lobed and finally divide still farther. Still more embryos may result from a later division of some of the larger multicellular embryos of the lower group. This late division of the embryo is designated as bud formation or budding, in order to distinguish it from the cleavage polyembryony which results from the component parts of the early embryo system. These divisions may be due to a division of the apical cell, resulting in two groups of cells appearing as two unequal or nearly equal lobes. Several cases of budding were observed, although usually the embryos do not form buds but appear as in fig. 12. Fig. 13 shows an instance in which the two lobes were nearly equal in size. Presumably this might result in the development of two embryos if the two should be in the terminal position. In this stage it is still possible to determine that this is a case of budding, since this secondary suspensor of embryonal tubes may be traced back to a common origin from a primary suspensor attached in turn to the remains of the prosuspensor. If two separate embryos had grown together to give this appearance, their secondary suspensors would be expected to separate easily and trace back to separate primary suspensors, showing an independent origin of the two embryos.

Fig. 19 seems to be an instance of twinning which resulted from the development of two bud lobes coming from a division of a single embryo. The two embryos were on the end of a common, closely interwoven secondary suspensor, as indicated by an examination and dissection which was carried out after the drawing was made. The prosuspensor of neighboring zygotes may usually be disentangled and may easily be separated by dissection.

In these later stages, however, when two buds of equal size are attached to the same secondary suspensor, they have an opportunity to develop farther into the later stages, if they occupy a terminal position among the embryos.

Whether formed by this later stage of budding or by the earlier polyembryonic cleavage, the embryos in fig 19 would probably represent identical twins. Considering the usual slight inequalities of two closely associated embryo systems such as those of figs. 7 and



FIGS. 16-19.—Figs. 16, 17, external appearance of embryos at formation of cotyledons. Fig. 18, mature embryo with two cotyledons, as dissected from seed nearly ready to be shed. Fig. 19, twins dissected from similar seed showing connection by means of common secondary suspensor, *k*, margin of root cap; $\times 25$.

8 (due to unequal development of the prosuspensors), it is unlikely that two embryo systems would contribute two fully developed embryos to the combined polyembryonic complex. There is, therefore, great probability but not absolute certainty that two embryos found matured in the seeds of *Sciadopitys* represent identical twins. Fig. 19 represents a case of identical twins even though they are

unequal in size. When two embryos survive to this stage, it is probable that they both germinate, as they occasionally do in some of the large-seeded pines (12). The theoretical possibility of identical twins in conifers may be of interest, but the mechanism of embryonic development is so designed that the survival of more than one embryo is only the rare or exceptional condition. From dissections of about forty embryos in stages between figs. 16 and 18, only one case of two embryos with cotyledons was observed. This is the one shown in fig. 19; however, there were usually many small embryos pushed up above the suspensor of the older embryo. These range in size from the one shown above in fig. 17 to the size of fig. 12 (represented as thrice the magnification of figs. 16-19) and smaller.

Order of differentiation of embryonic tissues

No distinct differentiation of tissues was found, other than that of embryonic cells and the massive secondary suspensor of embryonal tubes in the stages of figs. 11, 12, and 14. Between the stages of figs. 14 and 15, the arched internal arrangement of cells forming the plerome apex of the root becomes recognizable. In *Pinus* (2) the stem tip meristem becomes visible soon after the plerome apex of the root, although it develops into only a slight protuberance and is soon followed by a ring of cotyledons surrounding the stem tip. As shown in fig. 17, no such protuberance marking the stem tip meristem appears before the two cotyledons are formed. Embryos of the size represented by figs. 18 and 19 are still without a visibly organized stem tip meristem. The stem tip is probably delayed until after the seed begins to germinate. This condition was found in *Cephalotaxus* (5), and differs from all the Abietineae (2, 3), where a more or less distinct cone usually marks the meristematic bud of the stem tip, appearing before the cotyledons and long before the embryo is half grown. Two cotyledons are usually formed from as many broad primordia; occasionally three have been found. Out of 36 embryos with cotyledons or well formed primordia, three were found which had three cotyledons. Figs. 18 and 19 show a slight depression (*k*) surrounding the root end of the embryo. This marks the marginal edge of the root cap. *Pinus* does not show such a distinct depression externally, but the internal cell arrangement shows

where the root cap begins, and the root cap embraces about one-half the length of the embryo axis. On the other hand, *Cedrus* and also *Keteleeria* (11) have a shorter, more distinct, and similarly marked root cap which is recognizable in an early stage by the shape of the embryo (3). In *Sciadopitys* the root cap is of much smaller proportions than in *Pinus*. It includes less than one-fourth the length of the embryo, while in *Pinus* it may include nearly half the length of the embryonic axis. In both *Pinus* and *Sciadopitys*, and in conifers generally, so far as has been observed, the root cap is continuous with, and merges gradually into, a massive secondary suspensor. These internal anatomical features will be described elsewhere in greater detail.

Discussion

The embryogeny of *Sciadopitys* represents a somewhat generalized and rather variable type. Contrary to the opinion expressed by LAWSON (17), certain features have been found in the embryogeny which relate it to that of the Taxodineae, Cupressineae, and several other conifer lines. Cleavage polyembryony was the rule from which no exceptions were found in an examination of many embryo systems. The number of embryos formed per zygote greatly exceeds the usual eight embryos of *Pinus* and *Cedrus*, but these three genera do have in common the characteristics of cleavage polyembryony, an apical cell stage in the individual embryos, and rosette cells and embryos. Aside from these features, the embryogeny differs distinctly from that of the Abietineae (2, 4, 7).

The proembryo of *Sciadopitys* undergoes at least one more free nuclear division than the Abietineae before wall formation. According to the partial description of the proembryo given by LAWSON, the terminal group of cells, or the lowest tier as he calls it, organizes into more than twelve cells instead of the four found in the lowest tier of the Abietineae. It may be presumed that these cells represent embryo initials, and that their organization is not completed even after walls begin to appear in the proembryo. In this feature the proembryo differs from that of *Pinus*, where the four primary embryo initials appear to be organized when the first cell walls are laid down, followed immediately by formation of the tier of rosette embryo initials.

The sixteen or more species of *Pinus* which have been studied showed no budding whatever in a later stage. The more mature embryo of *Sciadopitys* also differs from that of *Pinus* in the length of the root cap, the number of cotyledons, and the time of organization of the small protuberance which represents the stem tip. The embryogeny of *Sciadopitys* therefore shows a great divergence from that of *Pinus* in its later stages; and in the early stages it has only the tier of rosette cells, cleavage polyembryony, and the apical cell stage as features in common. The prosuspensor of *Sciadopitys* probably has no corresponding structure in the embryogeny of *Pinus*, except in the rare cases where rosette cells elongate (2). The embryonal tubes or additions of secondary suspensor cells in *Pinus* are usually formed differently, since the apical cell has, for a longer period, a single cutting face.

The Cupressineae do not differ greatly from *Sciadopitys* in their embryogeny. If *Biota* and *Libocedrus* (6) are somewhat representative of the Cupressineae, the points of agreement embrace such features as the formation of a prosuspensor, but there seem to be no rosette cells. *Biota* differs from *Sciadopitys* in having a relatively long period of primary suspensor elongation during which the terminal cell remains inactive (6). In *Sciadopitys* the embryonic cells divide soon after the primary suspensors begin to elongate, and may divide to form several cells before elongation. So far as observed, *Biota* agrees with *Sciadopitys* in the position of the cell walls of the early divisions. It also has budding in the later embryo and usually two cotyledons. The rosette cells of *Sciadopitys* might afford a difference from the conditions in the Cupressineae, as their proembryos and early embryos have been described (6, 16, 18, 19, 20, 22), but since *Sciadopitys* was found to vary considerably, having no rosette cells in about one-third of its embryo systems and only a few scattered rosette cells in nearly half, the general conditions may still be considered in essential agreement. The prosuspensor cells of the Cupressineae are fewer in number than in *Sciadopitys*, as are also the number of free nuclei formed in the proembryo. On the other hand, the archegonial complex of the Cupressineae constitutes an organization not found in *Sciadopitys*, and the resulting smaller archegonia may offer a feature with which a reduction in the number

of free nuclear divisions in the proembryo is in some way connected. Likewise the number of embryo initials represented per zygote may have been conditioned by these changes in the transition from a relatively large embryo resembling *Sciadopitys* to a smaller one resembling the Cupressineae. This suggestion is further strengthened by the fact that in the Callitroideae, represented by *Callitris* and *Actinostrobus* (18, 19, 20), which were doubtless derived from the Cupressineae, the number of archegonia is increased still further, and the number of free nuclear divisions is reduced to the condition of wall formation after the second division of the zygote. It is obvious that the transition from *Sciadopitys* to certain of the Cupressineae as we know their embryogenies is not difficult. It seems, in fact, a very natural step in the evolution of conifer embryo types.

The relation which holds between the type of embryogeny found in the Taxodineae and *Sciadopitys* is not so obvious at first sight. COKER's (8) investigation shows that cleavage polyembryony is found in *Taxodium*, and that there are no rosette cells. (COKER uses the term rosette to designate the free nuclei in the archegonium above the prosuspensor, but others have used this term in designating a group of cells with cell walls in this position.) His study of *Taxodium* indicates that a brief stage of apical cell growth may be identified in the earliest stages. The prosuspensor cells may become somewhat separated from one another, but appear to persist and grow very long with one or sometimes two embryo initials or cell groups attached to their ends. When embryos become multicellular, an irregular fringe of embryonal tubes appears at their margins, projecting divergently backward on all sides of individual prosuspenders. The primary suspensor appears to be relatively short and sometimes omitted, by which is meant a suspensor cell which becomes elongated singly between prosuspensor and embryo. At least it appears that if the primary suspensor is not omitted, it is short and may not be distinctly recognizable from the other embryonal tubes which follow by elongation in close succession. This interpretation, based in part upon my own unpublished observations of *Taxodium*, is in agreement with COKER's account, and brings these embryogenies into sufficient agreement with *Sciadopitys* to permit their derivation from this general type. The archegonial complex, which is also

found here, has probably conditioned this transition to the Taxodineae much as this structure and the smaller archegonia seem to have influenced the transition to the Cupressineae: fewer free nuclear divisions in the proembryo, fewer embryo initials per zygote, and fewer cells in the prosuspensor.

Passing to *Sequoia* (1, 13), which appears to stand higher and is placed in a separate subfamily by some botanists, we seem to have smaller and still more numerous archegonia, with no free nuclei in the proembryo, and only a few embryo initials organized so far as known, with only one of them remaining functional on the end of a single-celled prosuspensor. That this parallelism in the evolution of embryogenies has come about in the two lines Cupressineae-Callitroideae and Taxodineae-Sequoideae, where a terminal archegonial complex appeared which involved more and more eggs and finally became lateral, is an extremely interesting situation, which calls for further consideration as other embryogenies in these two groups are investigated. It seems obvious that events connected with the history of the archegonial complex and resultant reduction in the archegonial size have had something to do with the decrease in the number of free nuclei in the proembryo, the reduction in the number of embryo initials per zygote, and the number of cells in the prosuspensor.

In its appearance and organization, the early embryo of *Sciadopitys* relates itself satisfactorily to that of *Cephalotaxus* (5, 9, 15, 22). The rosette group and terminal dead cap cell of *Sciadopitys* (figs. 1-5) might be substituted for the embryo of *Cephalotaxus*. The cap cells of the latter are larger, and usually several aborted cells are involved in the cap; otherwise there is no essential difference. In *Cephalotaxus* all embryo initials represented below the prosuspensor combine to form a single embryo, but the rosette cells form small separate embryos which have suspensors of embryonal tubes but no primary suspensor (5). An important essential difference between these two types is therefore to be recognized in cleavage polyembryony. In *Sciadopitys*, 12-15 embryos may come from the group below the prosuspensor, one of which contributes the embryo of the mature seed; in *Cephalotaxus* the lower units combine and only a single embryo comes from this region, and those of the rosette region

are more constantly present, persist longer, and develop to an older stage than do those in *Sciadopitys*. Another essential difference between these two types is in the apical cell growth. In *Sciadopitys* apical cell growth may be recognized; in *Cephalotaxus* an apical cell has not been observed, either in the single primary embryo or in the rosette embryos. A difference may possibly be found also in the time of wall formation in the proembryo, since it is not certainly known for *Sciadopitys* whether only eight or more than eight free nuclei are formed before walls appear in the proembryo (17).

Podocarpus spicatus has an early embryo which resembles that of *Sciadopitys*, at least in general organization (6, 21). In *P. spicatus*, however, in common with all species of *Podocarpus*, *Dacrydium*, and *Phyllocladus*, binucleate cells are found which represent embryo initials (6). Rosette cells which early become aborted are sometimes present, and the terminal binucleate cap is usually a single cell. While *P. spicatus* otherwise resembles *Cephalotaxus*, it has more completely eliminated cleavage polyembryony, producing only a single embryo from the group of cells below the prosuspensor, and no rosette embryos from the few scattered rosette cells. It appears, therefore, that *Sciadopitys* has an early embryogeny which may be considered generalized rather than specialized. It may serve to illustrate a starting point for the embryogenies of both the Taxodineae and Cupressineae. Before cleavage of the zygote, it is so similar in general organization and appearance to the embryos of *Cephalotaxus* and certain podocarps as to suggest a common origin, and it shares several features in common with the embryogeny of *Pinus* and the Abietineae, to which it is more remotely related. This suggests that *Sciadopitys* occupies a central position in the phylogeny of certain groups of higher conifers, remaining near the plexus of several diverging lines of evolution.

Summary

1. The early embryo of *Sciadopitys* shows two or three regions or tiers of cells which may develop into later stages. The cells of one of these regions elongate to form the prosuspensor; those of another represent embryo initials situated below the prosuspensor; and those of the third, which may be present, are rosette cells above the prosuspensor. A cap cell consisting of a terminal aborted embryo

initial may be present, and other embryonic cells anywhere in the embryo system may become aborted.

2. As the prosuspensor cells become fully elongated, the embryo initials give rise to embryos which are pushed out on single-celled, primary suspensors. The early embryos pass through a stage in which an apical cell is recognizable. Rosette cells, when present, are embryo initials. These may give rise to embryos which are usually aborted in early stages.

3. Embryonal tubes are formed which reinforce the primary suspensor, thus giving rise to a multicellular secondary suspensor.

4. The potential output per archegonium is 12-28 embryos, which may develop independently for a time. If three eggs are fertilized, an embryo complex may be formed with several times this number of separate embryos.

5. Embryos may undergo budding and twinning in later stages; however, the seed usually contains only a single embryo which is fully matured with cotyledons. The number of cotyledons is usually two but occasionally three.

6. The stem tip is not organized until long after the cotyledons are formed.

7. The root cap is relatively short, and merges very gradually into the secondary suspensor.

8. The embryo of *Sciadopitys* is a type from which the embryos of the Cupressineae may have been derived; it also represents a type from which the embryos of the Taxodineae may have been derived. It has probably had a common origin with the embryo type represented by *Cephalotaxus* and certain podocarps, and appears to be more distantly related to the Abietineae.

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[Accepted for publication May 29, 1931]

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AVAILABLE SOIL CALCIUM IN RELATION TO "DAMPING OFF" OF SOY BEAN SEEDLINGS

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(WITH TEN FIGURES)

Introduction

The prevalence of "damping off,"¹ with its large toll of plant seedlings, and even of plants, makes it a disease so serious as to invite study of any plausible suggestion toward its prevention. The plants which may be affected are not limited to any species, genera, or even family. STEVENS and HALL (9) state that any kind of plant is susceptible. Fungi have commonly been considered the pathological agency, with emphasis on *Pythium de Baryanum* and *Rhizoctonia* (6). although the list includes *Fusarium*, *Botrytis*, *Corticium*, *Thielavia*, *Sclerotium*, *Phytophthora* (9), and numerous others. Attention has been given to environmental conditions as factors (4, 9, 5), placing importance on high temperature and high moisture as favoring development of the disease. The soil has been considered as the habitat for the infecting microorganisms, but, so far as known, no attention has been directed to the possibility of the disease being associated with the physico-chemical properties of the soil.

In some studies attempting to isolate the effects of the soil calcium from those of the hydrogen-ion concentration upon inoculation and growth of the soy bean, the damping off of the seedlings occurred frequently in spite of complete sterilization of the seeds and the media. Variation in temperature or moisture conditions, the correction of crowding, the use of artificial ventilation, outdoor growth, and the exclusion of organic matter from the medium, all failed to prevent damping off. These facts, coupled with our recent knowledge of soil

¹ In this study, the term damping off refers to the dropping over of the young plant and other readily visible symptoms associated with the disease usually designated by that name. No study of the infecting fungi, internal plant structure, or other conditions was made. No claim is advanced that the disease in this study is the exact disease of this name by other investigators. For this reason throughout this paper the term damping off should be read as in quotation marks.

colloidal behavior (1, 8), suggested the hypothesis that physico-chemical conditions centering around the calcium and hydrogen-ion concentrations of the soil might also be responsible for this disease.

Methods

The plan of testing calcium and pH as factors influencing damping off necessitated careful control of the physico-chemical conditions of the soil or substrate. As a growth medium, a mixture of quartz sand, calcium clay, and other calcium compounds was used. The quartz sand was first treated with strong hydrochloric acid and then extracted with water until free from chlorides and until neutral to bromthymol blue. A natural clay was obtained from the fresh sub-soil of a Putnam silt loam by separating out the colloidal fraction by means of the super centrifuge. The size of the particles of this clay varied, according to BAVER (2), from 121 to 136 m μ . Analysis showed that it contained primarily adsorbed calcium and hydrogen ions, and that it had a molecular ratio of silica to alumina of about 3 to 1.

In order to eliminate unknown variables in the composition of the clay, this material was subjected to rigid purification by electro-dialysis, according to the method of BRADFELD (3). This method removes particularly those ions which are adsorbed² in exchangeable form on the surface of the clay particle. Clay so prepared contains for practical consideration only H-ions in exchangeable form. It is acid to a degree as low as pH 3.5, depending on the clay-water ratio, and permits varying degrees of substitution of the adsorbed H-ion by any other cations. These are also held by the clay particle in exchangeable form. The entire system is well buffered.

For the preparation of the calcium clay, varying quantities of calcium hydroxide were added to the pure hydrogen clay. The changes in degree of acidity in consequence of this treatment, as determined by the hydrogen electrode, are given in the titration curve of fig. 1. This curve served as the basis for the preparation of clays of varying hydrogen-calcium ratios, and consequently of vary-

² In using the term adsorption, it is intended merely to state that the ions are held on the surface of the colloidal particles; no attempt is made to distinguish between the chemical or physical nature of the forces responsible.

ing amounts of exchangeable calcium. Clay so prepared was mixed with sand in proportions never exceeding 2 per cent clay, and afforded excellent textural conditions.

Since adsorbed calcium enters into solution only when exchanged or replaced by other cations (including H-ions), it was deemed advisable to compare this clay-calcium compound with solutions having free, diffusible ionic calcium of the same concentration. Calcium acetate and calcium chloride were used for this comparison. Calcium acetate of various degrees of acidity was prepared according to the titration curve in fig. 1. No other nutrients were added to the media, since colloidal soil studies have demonstrated that the addition of ions to colloidal clay brings about, through ionic exchange and adsorption, entirely new conditions (7). The plants were limited to the nutrients in the seed and the media. This method of using the clay to supply the calcium limited the amount of this element to that quantity released from the clay by plant action.

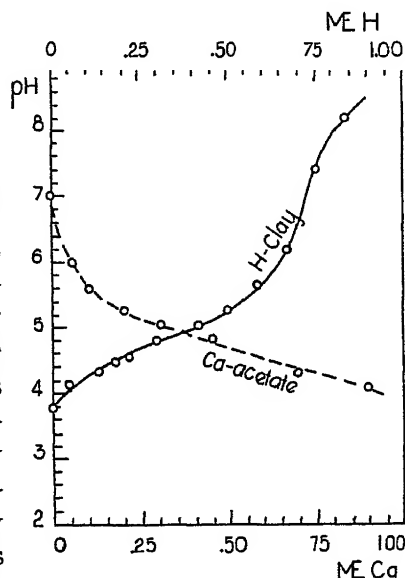


FIG. 1.—Titration curves of H clay + calcium hydroxide and Ca acetate + acetic acid as bases for preparation of media (ME Ca and ME H, milliequivalents of calcium and hydrogen, respectively).

Investigation of damping off

SYMPTOMS.—The symptoms of damping off as noted were a browning of the stem area just above the sand surface, the stem becoming soft, flaccid, and slimy, with a final breaking over of the plant. In some cases the degree of browning was less marked, the plant remained standing, became stunted, withered at the top, developed many adventitious buds at the cotyledons, and later the stem above this point fell over. After the first signs of the disease,

counts of the afflicted plants were taken at regular intervals until time of harvest.

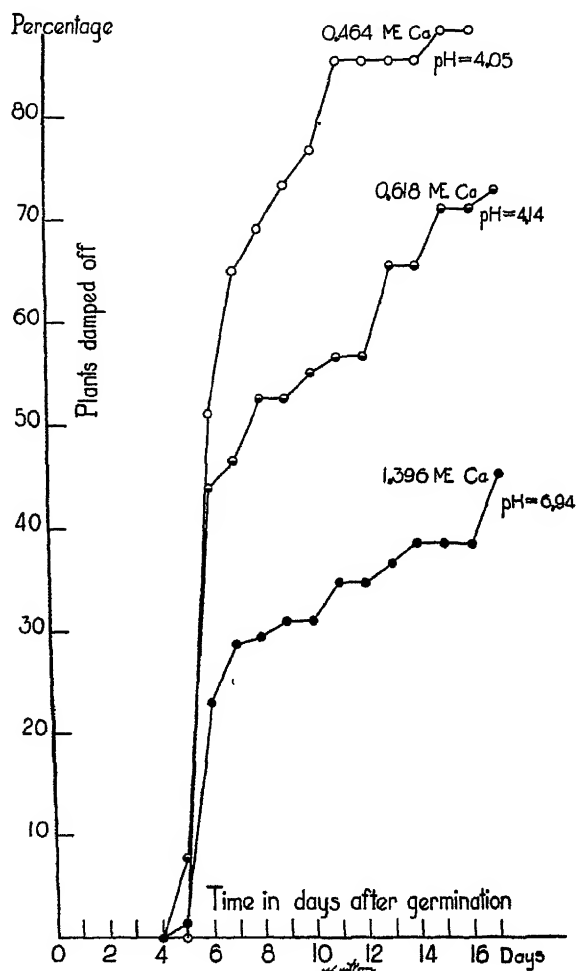


FIG. 2.—Increase of damping off with time: upper curve, low calcium content (0.464 ME per 65 plants) highly acidic (pH 4.05); middle curve, higher calcium content (0.618 ME per 65 plants) less acidic (pH 4.14); lower curve, high calcium content (1.396 ME per 65 plants) neutral reaction (pH 6.94).

RELATION TO TIME.—In all cases investigated it was found that damping off showed a definite relation to time (fig. 2). After germi-

nation between moist towels (root tips being 1-3 cm. long), the seedlings were transferred to glass jars with sandy medium. The plants grew vigorously for a few days, but after a week damping off occurred very noticeably, in that a great number of plants became afflicted suddenly and died within a short time. With increasing time, the rate of damping off gradually lessened, bringing about a curve which makes an asymptotic approach to the maximum of damping off for each particular treatment.

EFFECT OF VARIATION OF BOTH CALCIUM AND HYDROGEN IONS.—In order to simulate conditions of natural clays of humid regions, which contain primarily calcium and hydrogen ions in the adsorption complex, the first part of the study used constant amounts of clay but varied the amount of calcium and hydrogen ions on the clay particles. For every increase in calcium, there occurred a corresponding decrease in acidity (hydrogen ions). Two different experiments were run, extending from May 2 to July 15, and from July 10 to August 10, 1929. Glass jars with 1000 gm. of sand, each containing the necessary amounts of 1 per cent clay sol in one series and 0.052 normal calcium-acetate solutions in another series (for comparison), were planted each with 65 sterilized soy bean seeds. The water content of 20 per cent was maintained constant by daily weighings and water additions. Glass tubes were inserted vertically to facilitate aeration. Other environmental conditions were maintained as uniform as possible under greenhouse conditions.

The first experiment increased the calcium in colloidal clay and as calcium acetate from 0 to 2.15×10^{-2} milliequivalents per plant through ten jars by increments of approximately 0.24×10^{-2} , and decreased the hydrogen correspondingly from 2.3 to 0.15×10^{-2} through a pH range from 3.78 to 6.94. The results of these trials are given in fig. 3. In the second experiment, in order to use larger amounts of calcium, it was increased in these same two forms from 2.3 to 20.9×10^{-2} milliequivalents per plant through five jars by increments of approximately 4.6×10^{-2} , while the hydrogen decreased from 18.6 to 0×10^{-2} through a pH range from 3.8 to 8.0. Here again the clay series suffered more damping off than the acetate series. The range of the affliction was from 29 to 5 per cent for the clay series and from 14 to 0 per cent for the acetate series, decreasing in each with increasing calcium.

The most noticeable result of these two trials is the fact that, within the range in amounts of calcium and hydrogen ions used, the damping off decreased as acidity decreased and calcium increased. This is so, as shown in fig. 3. whether the calcium is supplied in adsorbed form by calcium clay, or in ionic form as calcium acetate. The percentage of injured plants was lower for calcium acetate than

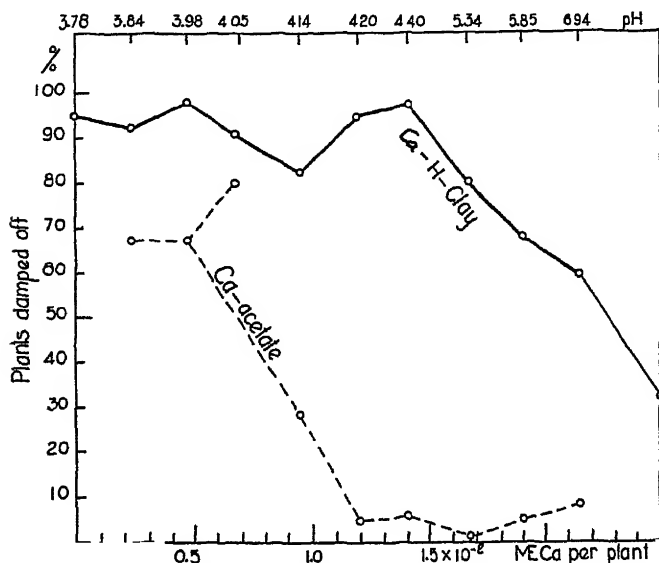


FIG. 3.—Decrease of damping off with falling acidity and increasing calcium concentration.

for calcium clay (fig. 4). This difference was less marked, however, and tended to disappear, as the ratio of hydrogen ions to calcium ions approached zero (fig. 3). It is interesting to note that in the second experiment, where more clay and consequently more hydrogen and calcium ions per plant were supplied, the percentage of damping off at any pH was considerably less than in the first trial, although in both cases the hydrogen-calcium ratio remained unchanged. The main significance of these trials undoubtedly lies in the fact that the observed damping off is definitely associated with the nature of the cation adsorbed on the colloidal complex.

Since the decrease of damping off resulted from variations in

amounts of both hydrogen ions and calcium, the causal effect cannot be specifically ascribed to either the calcium deficiency or the hydrogen-ion excess. In order to isolate the specific influence of the two variables (Ca and H), it was necessary that one factor be kept strictly constant while the other was varied.

EFFECT OF HYDROGEN IONS ON DAMPING OFF AT
CONSTANT CALCIUM CONCENTRATION

I. LOW CALCIUM CONCENTRATION.—In the third trial an attempt was made to isolate the separate effects by the hydrogen ions on

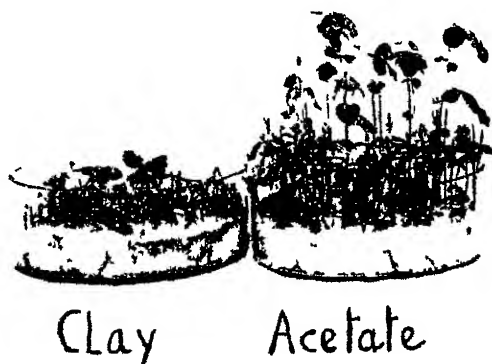


FIG. 4.—Damping off more pronounced with Ca clay than with Ca acetate (pH 4.05, Ca concentration 0.72×10^{-2} ME Ca per plant in both jars).

damping off. Calcium was kept constant but at a low concentration. Recent physico-chemical investigations of soils have shown that the colloidal clay fraction has a certain saturation capacity for cations. Consequently the total ions to be adsorbed and their ratio to each other are determined by this saturation capacity. There is then an upper limit in the adsorption of calcium for a given amount of clay. Below this limit the amount of calcium may be varied, but it is accompanied by an inverse variation in the hydrogen, or gives a varying hydrogen-calcium ratio. In order to increase the hydrogen-ion content of the medium beyond this limit, larger amounts of clay must be added.

A series of nine pots was used, containing in every case the constant amount of 0.0146 milliequivalents of calcium per seed in the

form of calcium clay. The acidity varied from pH 3.8 to 6.94. In order to obtain the necessary constant calcium concentration at low pH (wide H:Ca-ratio on the clay particle), larger amounts of clay per seed had to be added to the sand. The amounts did not exceed 2 per cent of clay in the sand. These trials extended over the same time as the preceding ones and were conducted under similar care and conditions. The data of the numbers of diseased plants for the

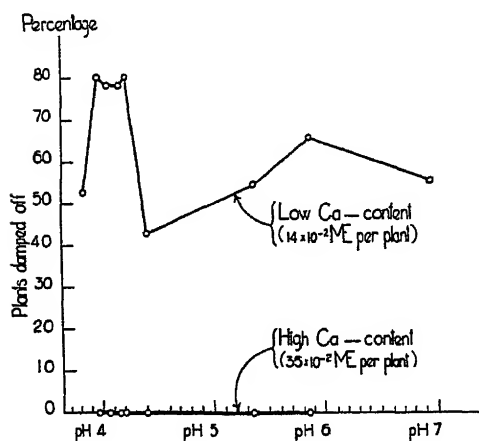


FIG. 5.—Negative effect of pH of Ca clay on damping off in presence of low and high calcium content.

various pH figures are given in the upper graph of fig. 5.

For these low amounts of calcium, no definite correlation was observed between the percentage of diseased plants and clay acidity. The number of dead plants varied from 43 to 80 per cent, irrespective of whether the reaction of the medium was neutral or acid. Even near pH 7.0, where the hydrogen-calcium ratio approaches zero,

over 50 per cent of the plants died. This suggests that under constant, low calcium concentration the disease is not controlled by the amount of hydrogen ions present. In this trial with low calcium application there was a marked manifestation of the disease independent of the hydrogen-ion concentration. This, however, does not necessarily guarantee the indifference of the disease to the hydrogen-ion concentration at other concentrations of calcium, nor does it establish a causal relation between the concentration of calcium and the disease.

II. HIGH CALCIUM CONCENTRATION.—In the next trial, a higher concentration of calcium, 0.350 milliequivalents of calcium per seed or an increase of 24 times, and a varying acidity of pH 4–6 were used. This was done by using clay suspensions like the previous,

but by supplying in every case larger amounts of clay. Each plant was grown in an individual container (300 gm. sand) in order to prevent the use of mineral excretions from dying plants by those remaining healthy. Each concentration of calcium was replicated three to ten times. This trial extended from November 22 to January 10, under conditions and manipulation corresponding to those of the preceding trials. The plants were observed daily for the possible development of the diseased condition. The records are given in the lower graph of fig. 5, and may be compared with the data for lower Ca concentrations in the upper graph.

Again at this particular calcium concentration, there was evident no relation between the disease and the hydrogen-ion concentration. But, contrary to the previous experiment at low Ca concentration, not a single plant showed any indication of the disease. This indicates clearly that the hydrogen-calcium ratio (pH) is not a factor in the prevalence of the disease when the supply of calcium is either low or high. It suggests, however, that the disease is related to the second variable, namely, the calcium.

EFFECT OF CALCIUM ON DAMPING OFF AT CONSTANT pH

In order to test the importance of calcium, there was set up a series of increasing clay concentrations and increasing calcium acetate concentrations, and therefore increasing amounts of calcium per seed, at constant but at two different hydrogen-calcium ratios. One set of plants was grown at about neutral reaction (pH 6.92 and 6.94) and one at acid reaction (pH 4.4).

I. INCREASING CALCIUM CONCENTRATION AT NEUTRAL REACTION.—One series of eight pans contained 65 plants per pan with variations of calcium from 0 to 4.2×10^{-2} ME Ca per seed, all at pH 6.94. The results are given in fig. 6. This experiment was carried out during the summer months of 1929. Another series, with 200 plants grown individually in nursery bottles containing a range from 0 to 35×10^{-2} ME Ca per plant at pH 6.92 lasted from March 15 to May 15, 1930, and gave the results according to table I.

These results, and those shown in figs. 6 and 7, are apparently conclusive as to the importance of calcium in connection with damping off in these trials. With increasing Ca concentration, the number

of diseased plants decreased in the case of both Ca clay and Ca acetate

TABLE I

DAMPING OFF AS INFLUENCED BY INCREASING AMOUNTS OF CALCIUM
AT NEUTRAL REACTION (pH 6.92), SUMMARY OF 200
PLANTS GROWN INDIVIDUALLY

MILLIEQUIVALENTS Ca PER PLANT	PERCENTAGE PLANTS DAMPED OFF
Ca-clay 0	100
0-5-5 × 10 ⁻²	29
6-20 × 10 ⁻²	7
25-35 × 10 ⁻²	3
Ca-acetate 0	100
5-10 × 10 ⁻²	25
30 × 10 ⁻²	0

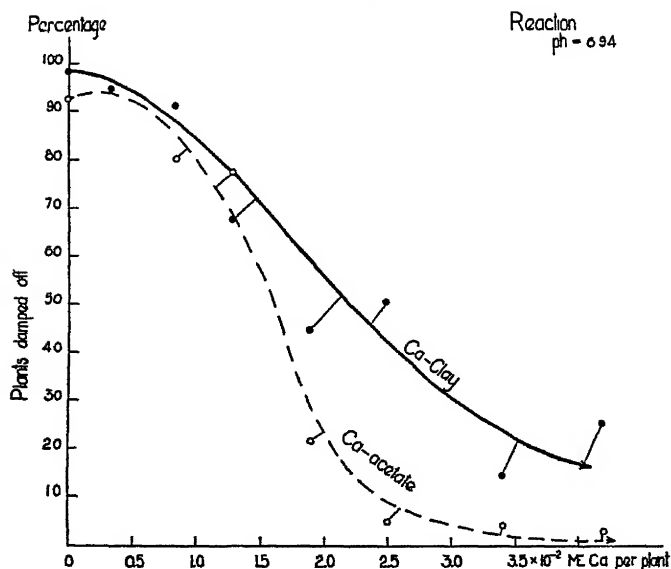
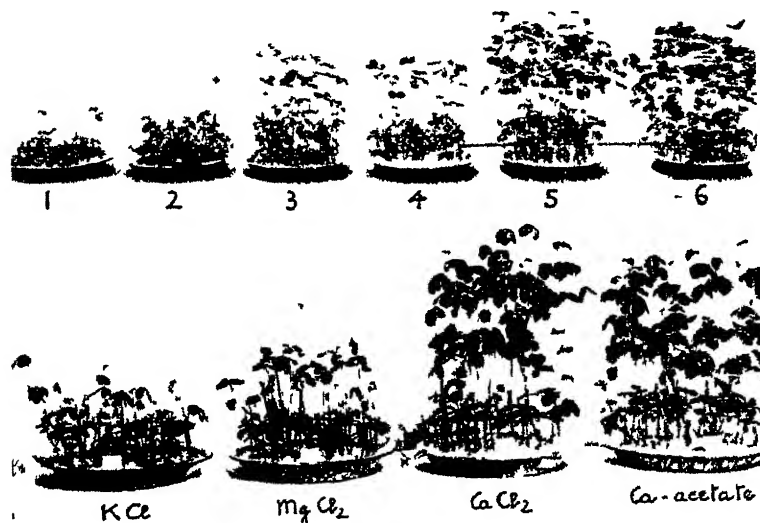


FIG 6—Decrease of damping off with increasing Ca concentration at neutral reaction

II. INCREASING CALCIUM CONCENTRATION AT ACID REACTION.—If calcium is a more important factor than acidity in controlling damping off, the preceding experiment should give similar results at

relatively low pH figures (acid reaction), and not only at pH values near the neutral point. A trial with increasing amounts of Ca ($0.86-3.08 \text{ ME} \times 10^{-2}$ per plant) at the very acid reaction of pH 4.4 was run during the months of June and July, 1930. The results are given in table II, and show that even at low pH the diseased plants decrease rapidly with increasing calcium concentration.



FIGS 7, 8.—Fig 7 (above), decrease of damping off with increasing Ca acetate concentrations ($0.34 \times 10^{-2} \text{ ME Ca}$ per plant) at pH 7. Fig 8 (below), effect of Ca as compared with that of K and Mg on damping off.

KCl	$10.8 \times 10^{-2} \text{ ME K}$ per plant
MgCl ₂	$7.8 \times 10^{-2} \text{ ME Mg}$ per plant
CaCl ₂	$8.4 \times 10^{-2} \text{ ME Ca}$ per plant
Ca acetate	$11.4 \times 10^{-2} \text{ ME Ca}$ per plant

In summarizing the preceding trials, the first observation, that damping off decreases as the hydrogen-ion concentration decreases and the calcium concentration increases, can be interpreted as follows: calcium ions are far more effective than hydrogen ions in controlling damping off. Trials with varying pH values at constant calcium concentrations (high and low) did not reveal any correlation between the disease and the hydrogen-ion concentration. There was observed, however, a close relationship between calcium content and

affected plants. This relation manifested itself independently of pH values.

COMPARATIVE IMPORTANCE OF CALCIUM AND OTHER CATIONS

In the preceding trials, the effect of calcium in controlling damping off was definitely related to the quantity of calcium present, when the medium contained only calcium and hydrogen. Not only the quantitative effect but also the qualitative importance of calcium in comparison with other cations can be demonstrated. Series of jars of 50 plants per 800 gm. sand were set up, one each for varying amounts of potassium chloride, magnesium chloride, calcium chlo-

TABLE II
DECREASE OF DAMPING OFF WITH INCREASING CALCIUM
CLAY CONCENTRATION AT pH 4.4

	LABORATORY NUMBER		
	¹ (a, b)	² (a, b)	³ (a, b)
Number of plants per pan . . .	65 0	65 0	65 0
pH (initial)	4.4	4.4	4.4
ME Ca $\times 10^{-2}$ per plant	0.86	1.92	3.08
Percentage damped off after 14 days . .	40	8	0

ride, and calcium acetate. These were handled in the same manner as in previous experiments.

For all salts used there was a decrease in damping off with increasing amounts of each salt. This decrease, however, was characteristic of each salt. For potassium chloride, the injured plants decreased from 94 to 74 per cent; in the case of magnesium chloride, the change was from 96 to 12 per cent; while for the calcium chloride and the calcium acetate the decrease dropped suddenly from 89 to 0 per cent as soon as a certain minimum concentration of calcium was reached. This minimum occurred at $2-3 \times 10^{-2}$ milliequivalents of calcium per seed. The difference in effects shown by calcium over that by the other cations is illustrated in figs. 8 and 9.

The trials thus far emphasize the significance of calcium in connection with the occurrence of the disease. This significance is attached, first to the element calcium itself, then to the quantity of

calcium present, and finally to the nature of the anion with which it is associated (acetate, chlorine, and "clay"). The experiment also indicates that osmotic pressure of the medium is scarcely connected with the disease

AVAILABILITY OF CALCIUM AND ITS RELATION TO THE DISEASE

The observation that calcium acetate is somewhat superior to calcium clay in preventing damping off, suggests that the availabil-

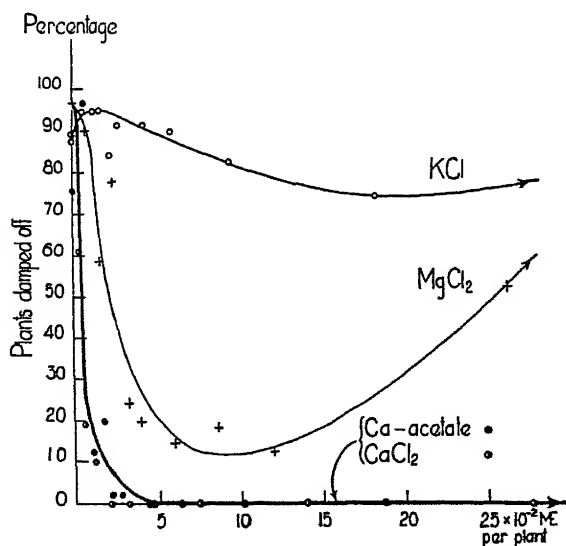


FIG. 9.—Specific effect of calcium in preventing damping off as superior to that of potassium and magnesium.

ity of calcium might also be of importance in controlling the disease. The following Ca carriers were selected to test the validity of this suggestion: (1) calcium acetate, which is highly dissociated and provides calcium in free, diffusable ionic form; (2) calcium permutit, in which case a commercial sodium permutit (an artificial aluminosilicate) was transformed into calcium permutit through continuous leaching with Ca chloride, providing calcium in adsorbed exchangeable form, that is to say, it can be set free through ionic exchange with other cations, particularly hydrogen ions (7); and (3) anorthite,

which is a natural aluminosilicate mineral, insoluble in either hydrochloric or sulphuric acid. Its calcium is held firmly in the crystal through the forces of the crystal lattice

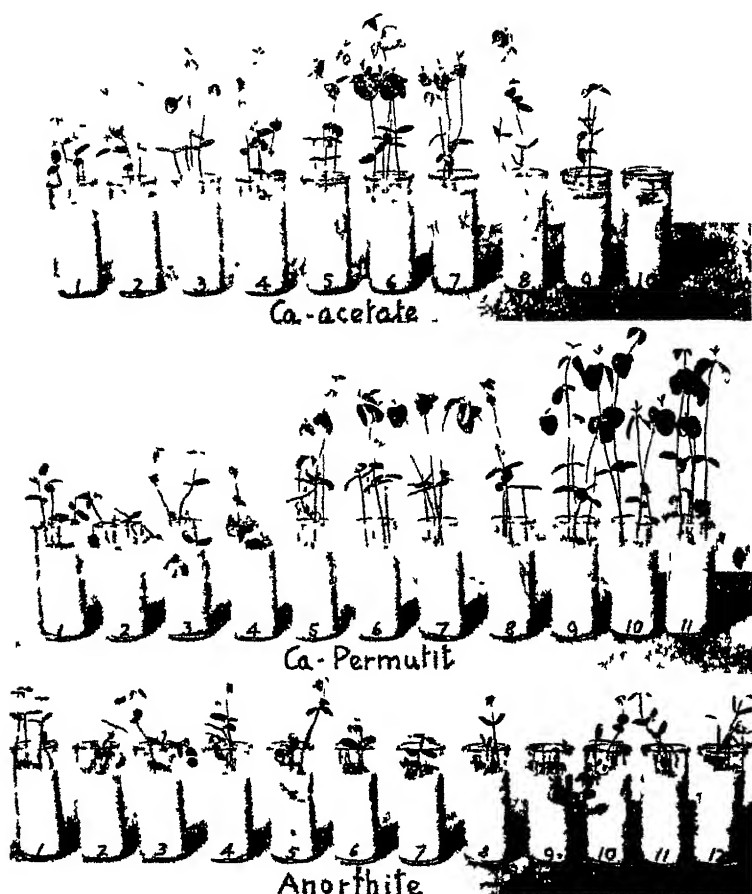


FIG. 10—Availability of calcium as factor controlling damping off. free, adsorbed, and crystal lattice calcium from upper to lower pictures; increasing calcium from left to right.

Plants were grown in nursery bottles in duplicate series containing 300 gm. of sand each and increasing amounts of calcium, varying from 1.25 to 250×10^{-2} ME Ca per plant (pH 7). Anorthite and permutit were ground and passed through a 40-mesh and collected

on a 60-mesh sieve in order to obtain particles of uniform comparable size. The treatment and results are shown in table III. Fig. 10 indicates the conditions after the tenth day of growth. The results obtained in these trials are striking. The plants grown on calcium acetate showed no signs of damping off, those on calcium permutit damped off at the lower but not at the higher concentrations; while the anorthitic lot was affected by the disease at all concentrations.

TABLE III
AVAILABILITY OF CALCIUM AS IT AFFECTS DAMPING OFF

	LABORATORY NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
ME Ca $\times 10^{-3}$ per plant	0	1.25	2.5	3.75	5.0	7.0	12.5	17.5	25	125	250	1250
pH (initial)	7	7	7	7	7	7	7	7	7	7	7	7
Number of plants per bottle	4	4	4	4	4	4	4	4	4	4	4	4
Number damped off after 14 days												
Ca acetate	1	0	0	0	0	0	0	0	0	0	0	
Ca permutit	1	3	2	3	3	0	0	0	0	0	0	
Anorthite	0	3	3	2	2	2	3	2	4	3	2	2

Evidently the availability of calcium is also a vital factor in the frequency of the disease. The effectiveness of ionic calcium in its power to influence the disease might be represented by the following series:

free, diffusable Ca > adsorbed, exchangeable, Ca > ^{fixed} Ca in the crystal lattice.

Summary

1. The damping off disease has usually been associated with unfavorable temperature, moisture, and light conditions, but these experiments show that, in the case of soy bean seedlings, the disease is also closely associated with the nature of soil colloids, particularly with the nature of the adsorbed ions (calcium).

2. Hydrogen ions are not an important factor in determining the disease. Within a range of pH 3.8-6.94 it was found that heavy damping off occurred when the calcium supply was low, but none occurred when the calcium supply was high.

3. Calcium is an important factor in the control of the disease. With increasing calcium concentration the number of diseased plants decreased rapidly, both at pH 7 and at pH 4.4. At high calcium concentration no damping off occurred between pH 3.8 and 8.0.

4. In preventing the disease, calcium ions are superior to other monovalent or divalent ions at equal concentrations. For the chlorides, the importance of cations in checking the disease decreases according to the series $\text{Ca} > \text{Mg} > \text{K}$.

5. Availability of calcium is an important feature in controlling damping off. Free, diffusible calcium ions were more effective than adsorbed, exchangeable calcium ions, while calcium bound by the forces of the crystal lattice in the mineral anorthite was not able to prevent the disease.

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[Accepted for publication April 16, 1931]

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CICATRIZATION OF FOLIAGE LEAVES

II. WOUND RESPONSES OF CERTAIN BROAD-LEAVED EVERGREENS

ROBERT B. WYLIE

(WITH SEVEN FIGURES)

Introduction

A recent article by the writer discussed the foliar wound responses of certain mesophytic plants and reviewed the more important literature in this field. This paper (3) dealt with the leaf organization and described both the preliminary and the secondary protective structures developed by wounded leaves of *Vitis vulpina*, *Rhus glabra*, and *Syringa vulgaris*. In these forms, as in all other mesophytes studied, there is a prompt collapse of dead tissues along the wounded margin following lesion, and the shrunken mesophyll, with the incurved epidermal layers, constitutes a protective buffer which has been termed the pseudocicatrice (2). Subsequent divisions of living cells underneath this preliminary barrier lead to the formation of the cicatrice proper, or specialized protective structure. This latter, which is developed slowly by the modification of living cells, consists in part at least of wound cork and is usually several layers of cells in thickness.

The present paper summarizes events following wounding of certain broad-leaved evergreens of the Pacific northwest. In 1925 the writer made preliminary observations on some of the evergreen angiosperms of western Washington. Further material was supplied by members of the botany staff of the University of Washington, in Seattle. The writer is under special obligation to Professor GEORGE B. RIGG of that institution for collections made in three successive years. During the summer of 1929 the writer spent several weeks at the Puget Sound Biological Station on San Juan Island, 60 miles north of Seattle, where certain experiments were carried out and further material was collected. This latter region is somewhat drier in summer than the Seattle area, precipitation being influenced by ad-

jacent mountains. The winters in the Puget Sound district are moist and relatively mild.

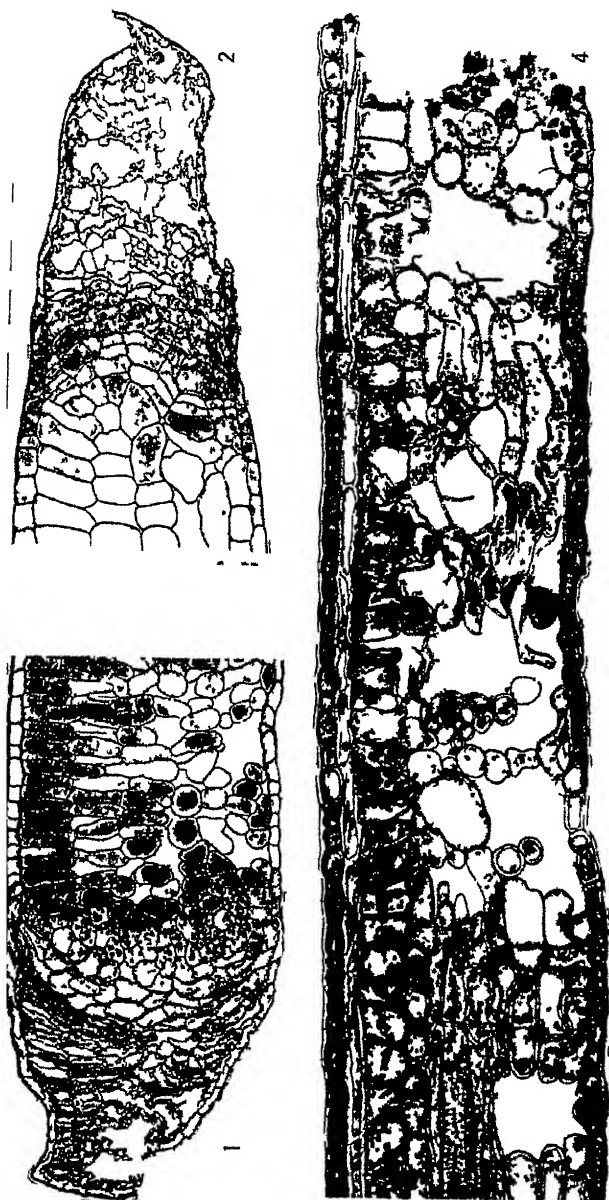
Following a preliminary study of a number of native broad-leaved evergreens of the Pacific northwest, three species were chosen for special study. Madrona (*Arbutus menziesii*), Salal (*Gaultheria shallon*), and Oregon grape (*Berberis nervosa*). These are all deep-rooted trees or shrubs with evergreen leaves which meet the problems of the changing seasons and function through two or more summers. Healthy leaves of typical plants were wounded during the latter part of June by cutting with scissors across the blades at right angles to major veins, and subsequently material was collected at intervals and preserved for critical study. Freehand and freezing microtome sections yielded general information, but for detailed study the tissues were imbedded in paraffin after preliminary treatment with hydrofluoric acid. Serial sections, cut both transversely to and parallel with the leaf surface, gave preparations for detailed study. The latter sections were especially helpful in interpreting the normal leaf structure as well as changes that follow wounds.

I. *Arbutus menziesii*

This peculiar tree with its beautiful foliage is one of the characteristic plants of the open shores from northern California to British Columbia. Each set of leaves lives somewhat more than a year in the Puget Sound region, where leaf fall occurs during the second summer (1). The oval leaves, with glossy upper surface, are about 75×125 cm in size, and average 285μ in thickness.

Transverse sections show a single upper epidermis with no suggestion of special subdermal development (fig. 1). In addition to a heavy cuticle, all walls of the epidermal cells are thickened, but numerous thinner areas in their lateral walls favor transfer of material within this layer. The upper cuticle is $3-4 \mu$ in thickness and about twice that on the under side, which has numerous protuberances that are almost spinous. The lower epidermis has much smaller cells and carries all of the stomata. The inner walls of the lower epidermis have also a well developed cuticle bordering on the large air spaces within the leaf.

The palisade is multiseriate, having usually two specialized layers of cells, while a third layer approaches mesophyll and is somewhat



FIGS. 1, 2, 3, 4.—Fig. 1, transverse section of *Arbutus menziesii* 40 days after wounding, showing pseudocuticle (collapsed) cicatrice, and normal leaf tissue; fig. 2, transverse section of *Gaillardia stricta* 40 days after wounding, showing pseudocuticle, cicatrice, and normal leaf tissue; fig. 3, transverse section of *Berberis nervosa* 10 days after wounding of mature leaf, showing normal leaf tissue, a few enlarged cells, and wide zone of dead tissue with fungal hyphae and spores (there has been no collapse of the dead tissue which occupies three fifths of this figure).

* All figures, except fig. 3, were prepared by using photomicrographs as found in the original sections. Photographs were made using a Zeiss microscope with a 16 mm. objective in combination with a no. 1 Homal. From these, negative, white, and, which were completed as drawings with microscopical study of the original sections.

intermediate both in position and character. The uppermost palisade layer, seen in section parallel with the leaf surface, has polygonal cells with small intercellular spaces at their angles. The second layer has cells more nearly cylindrical with proportionately larger intercellular spaces, while the third layer is still less compact with much more open space.

The loose spongy mesophyll resembles the palisade in that its cells are mainly vertical in arrangement, in contrast to the usual horizontal trend of cells in this zone. Sections parallel with the epidermis show some cell elongation in this direction, with a loose mesh arrangement only in the lower part of the leaf adjacent to the epidermis. The minor venation is rather uniformly distributed in the form of rectangular meshes which lie in the spongy mesophyll. The intervacular interval averages $227\ \mu$.

WOUND RESPONSES

The gradual collapse of dead mesophyll cells along the wounded margin leads to the development of a pronounced cushion, often 30 or more cells in depth. In addition to the partial barrier produced by this dead tissue, both upper and lower epidermal layers are drawn inward over the wounded margin of the leaf (fig. 1). This pseudocicatrice develops slowly through a period of several days but begins early its protective function. Subsequent enlargement of living cells underneath may separate somewhat the incurved epidermal layers, owing to the bulging growth of the cicatrice tissues.

Mitoses in living cells underneath the pseudocicatrice seem not to begin until about the tenth day following lesion. By the fifteenth day, the developing cicatrice involves a zone 4-6 cells across, and it is obvious that all cell layers of the leaf have contributed toward its formation through mitoses. A month or more is necessary for completion of the cicatrice. At maturity this barrier involves 6-10-cell layers of wound cork, and underneath are about as many more layers of living cells crowded irregularly together. The outer cicatrice cells enlarge considerably and have heavy walls on the outer convex side (fig. 1). The region of wound cork is usually somewhat swollen, increasing considerably the thickness of the leaf in the cicatrice region behind the pseudocicatrice. The preliminary barrier

is not displaced, however, as it is fixed in position by the overarching epidermal layers.

As early as the fifth day the cicatrice region shows positive reaction to phloroglycin tests for lignin, and during the following week this reaction becomes more marked. By the twentieth day the lignin seems to be restricted to a narrow zone outside the cicatrice proper. From the twentieth day on, all of the outer portions of the cicatrice stain with Sudan III, showing that this tissue becomes suberized as it matures.

II. *Gaultheria shallon*

In the region of study, San Juan Island in Puget Sound, *Gaultheria shallon* is usually a low shrub. Abundant in semiopen areas, it thrives with partial shade as a bushy undergrowth, but is often quite tall along forest borders. The large oval leaves are evergreen, about $250\ \mu$ in thickness, and usually live from two to four years (1). Transverse sections show a simple epidermis above and below, with about six interior layers of mesophyll cells (fig. 2). In addition to a single compact layer of short palisade next to the upper epidermis, there are one or two additional layers of isodiametric cells which are loosely arranged, leaving large intercellular spaces. The spongy mesophyll consisting of three or four layers has cells elongated and lobed in the horizontal plane, surrounding large air spaces. These tissues may be interpreted satisfactorily only from sections cut parallel with the epidermis. The veins are bordered above and below by heavy mechanical tissue, and the intervascular interval is $330\ \mu$.

WOUND RESPONSES

The exposed tissues along wounded margins collapse promptly but continue to die back slowly, so that it is several days before the pseudocicatrice takes its final form. The incurved cuticular layers may have completely folded over the wounded margin, unless prevented by adjacent supporting veins (fig. 2). By the tenth day, when cell enlargement begins in the region of the future cicatrice, there develops a sharp distinction between dead and living tissues. By the end of the second week several outer cell layers of the protected living tissue have enlarged until tightly pressed together, and their outermost walls have thickened considerably, but few cells have

as yet divided at this stage. In the spongy mesophyll the enlargement is most marked, with lobings and extensions of cells which bulge into the considerable spaces of this region. The developing cicatrice at this stage involves only 4-6-cell layers

By the twentieth day cell enlargement in the cicatrice region has become marked and some mitoses may have taken place. The enlarging cells become highly distorted as they push past each other into the spaces of the mesophyll, and outward against the yielding pseudocicatrice. By the fortieth day the situation has completely changed (fig. 2). Repeated cell divisions have established a cicatrice 10-20 cells thick. The outermost, derived from certain of the cells that enlarged earlier, are much compressed and pushed outward by the pressure of those later formed behind. The non-suberized interior cells underneath the cicatrice proper enlarge rather more than is usual for this region.

The cicatrice of *Gaultheria shallon* requires over a month for its completion, and cell divisions are delayed longer than in any other form studied; mitoses may be retarded until three weeks after wounding. In many cases the cells of the cicatrice are telescoped, the inner extending lobes into the outer. This situation is made possible by the local thickening of walls on the outer side of cells, leaving inner walls relatively thin and subject to compression on that side. The pseudocicatrice is so firmly anchored by the epidermal layers that it is not displaced by this pressure from the growing tissues underneath. In all later stages the cicatrice region reacts positively to tests for both suberin and lignin.

III. *Berberis nervosa*

The Oregon grape, while not abundant in the region of the San Juan Islands, is rather common in partly shaded areas and along forest borders. Its stiff compound leaves, although about the same thickness as those previously discussed, are firm and usually persist for several years (1). Since the mature leaf of *B. nervosa* growing in this locality is unable to develop a pseudocicatrice following wounding, its foliar structure was studied with critical interest. Failure to develop this preliminary barrier inhibits in turn the development of a true cicatrice. Ultimately the air spaces in a zone of varying

width are blocked by modified tyloses. Fungal hyphae are usually abundant in the wounded margins, and masses of spores are seen at the edge of the dead tissue (fig. 4) These unusual wound responses are correlated with certain structural peculiarities that have been noted only in the leaves of this species. Transverse sections through

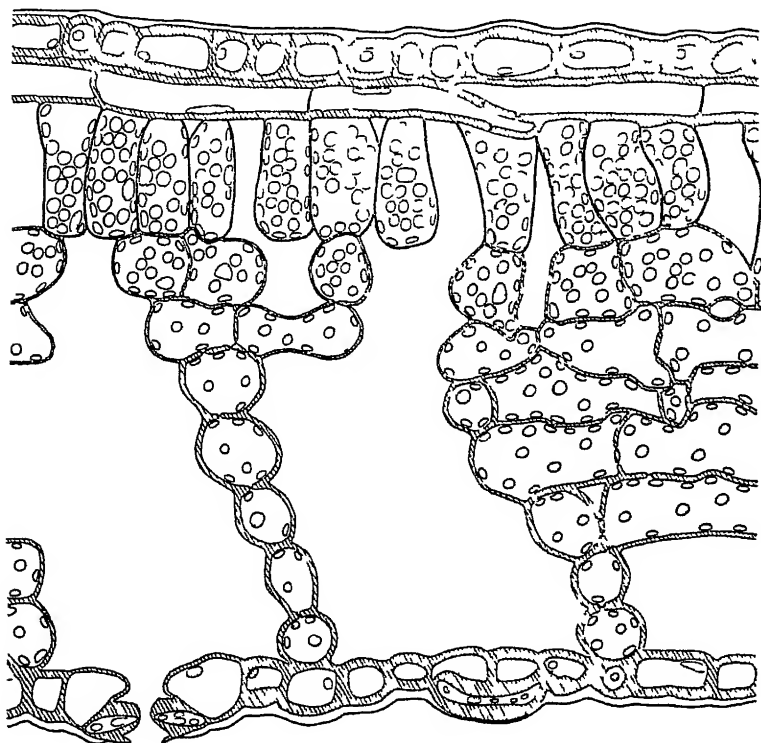


FIG. 3.—Transverse section of mature leaf of *Berberis nervosa*, heavy subdermal layer lies between upper epidermis and palisade, all cell walls much thickened.

the leaves show that this rigidity of structure is due primarily to the unusual thickness of all cell walls (fig. 3).

The sturdy epidermal layers, with heavy cuticular reinforcements, offer the usual specializations and have all stomata on the under side. There is also some cutinization of the inner surface of the lower epidermis, opposite the large intercellular spaces of the mesophyll. Next to the upper epidermis is a rigid subdermal layer of greatly

elongated thick-walled cells, which lie side by side in a compact layer continuous throughout the leaf area. A pinna dehydrated and cleared, thus permitting direct observation, showed that these cells are elongated parallel with the bases of the major veins, and continue this trend to the tips of the several lobes at the outer end of the leaf. This general direction is maintained in areas far removed from primary veins, and seems to be independent of the secondary venation. The cells of this mechanical layer are nearly square in cross-section, and are ten times as long as wide. The pitted walls are greatly thickened, and constitute a heavy structural reinforcement of the blade (figs 3, 4).

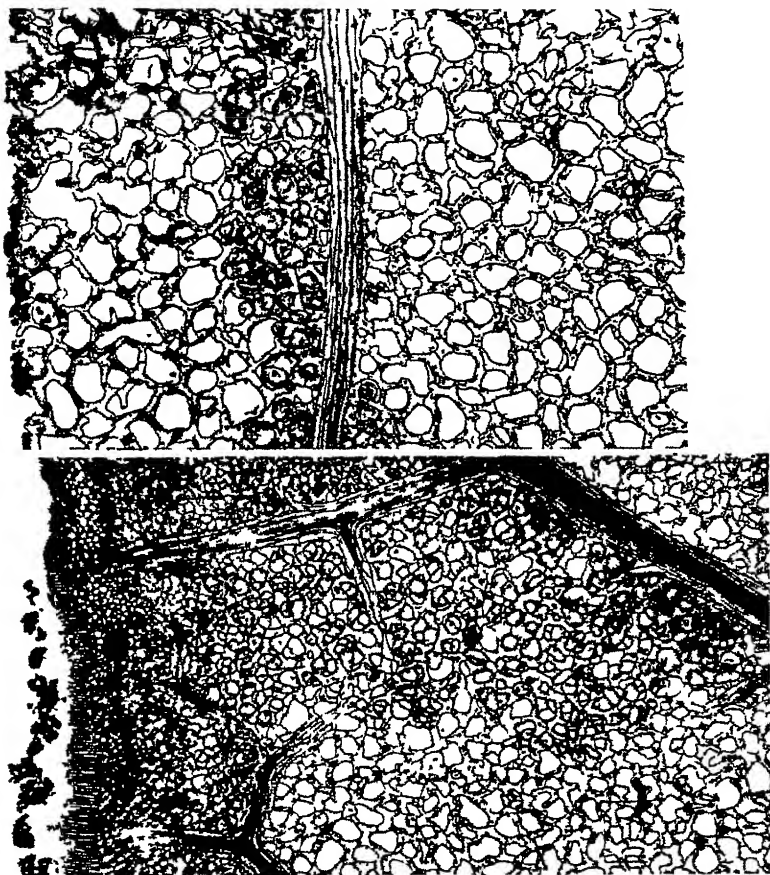
There is a single, simple palisade layer with cells about twice as long as wide, and underneath a layer of scattered squarish cells. This zone of the leaf has the thinnest walls and is especially rich in chloroplasts. The upper palisade layer, while rather compact, has spaces between its cells, and frequent larger openings lead up through it to the subdermal layer (fig. 3).

The five or six layers of spongy mesophyll have their cells elongated horizontally, and arranged in symmetrical meshes resembling the nets of *Hydrodictyon* but with a different cell arrangement (figs. 5, 6). These several layers have their meshes superimposed, surrounding relatively large air spaces, and appear in cross-section as columns of cells which tie the lower epidermis to the palisade region. This arrangement offers a degree of mechanical support not usually associated with the porous mesophyll. The intervascular distance is unusually great ($392\ \mu$); this wide space between veins is probably correlated with the reduced palisade and the horizontal elongation of mesophyll cells.

WOUND RESPONSES

The combined structural features of the leaf of *Berberis nervosa* make it peculiarly rigid. Not only are all cell walls unusually thick, but the spongy mesophyll is so arranged as to offer almost the rigidity of solid tissue. There are in addition the heavy-walled epidermal layers, and the leaf is further fortified on the upper side by the strong subdermal mechanical cells already described. This combination of structural features results in a leaf, which if mature when wounded, cannot collapse following lesion. Although the lifeless protoplasts

are shrunk within their walls along the wounded margin, there is no crumpling of any portion of the leaf tissue, all parts retaining their



FIGS 5, 6—Fig 5 (above), section of *Berberis nervosa* 40 days after wounding of mature leaf, cut parallel with epidermis and showing netlike arrangement of spongy mesophyll, dead tissue at left has fungal hyphae and spores, and zone of tyloses borders large vein on left side; normal leaf tissue at right Fig. 6 (below), section of leaf of *Berberis nervosa* 40 days after wounding of mature leaf, cut parallel with epidermis, dead tissue along margin is darkened by masses of fungal spores, and tyloses have extended far into leaf in region without larger vein.

original relations (fig. 4). Consequently there can be no formation of pseudocicatrice. In the absence of such protective barrier, the many

air spaces of the mesophyll remain wide open, although separated somewhat by the ranked and meshed arrangement of the cells. The exposed marginal tissues die back irregularly, usually to the region of the first important vein. This results in a dead zone of varying width (figs. 5, 6), in striking contrast to the usual rather uniform margin of lifeless tissue along the wounded edge. These dead mesophyll cells often contain considerable starch, which encourages the growth of certain types of fungi (figs. 4-6). Sections of living leaves show that the mesophyll is commonly used in summer for storage of starch, this being one reason for the relatively dull appearance of their under surface. In the sister species, *B. aquifolia*, the zone of dead tissue is nearly uniform in width, and the region of the well developed cicatrice runs parallel with the marginal pseudocicatrice.

It is of special interest that under the circumstances just discussed, which prevent the formation of a pseudocicatrice, there is no true cicatrice developed if leaves of *B. nervosa* are mature when wounded. Instead of a localized zone of wound cork, the intercellular spaces in a region of varying width behind the dead tissues are ultimately blocked by a system of tyloses formed from adjacent mesophyll cells (figs. 4-6).

Growth changes take place slowly in this species. By the fifth day there is a faint suggestion of lignin in the outer margin of living cells. On the tenth day, when cell enlargement is evident, the walls of this region react to tests for both lignin and suberin, the latter being much more pronounced nearer the epidermal layers. Both of these secretions persist in the region of wound response, even where there is little change in size and form of cells.

The tissue changes involved are limited practically to cell enlargement, first noted in the outer portion of living cells about the tenth day. Mitoses seem rarely to occur, although cells may continue to enlarge for three weeks. The width of the zone undergoing such modification varies greatly, and is related in a general way to venation. If a larger vein is near the wounded edge, its outer side is bordered by a narrow zone of greatly enlarged cells (fig. 5); on the other hand, if no important vein is near the wounded edge, there may be a wide region of marked cell enlargement (fig. 6).

The most striking developments are in the spongy mesophyll,

which is the most extensive of the interior tissues of these leaves. Living cells surrounding the meshes enlarge into the extensive intercellular spaces. Viewed in sections parallel with the epidermis (figs. 5, 6), it may be noted that the enlargements into spaces may be numerous; two or more may meet and flatten together like tyloses in the tracheal vessels of stems. These intrusive swellings, or tyloses, may be very large, amounting to many times the volume of the original cell of which each remains a part. Ultimately over a consid-

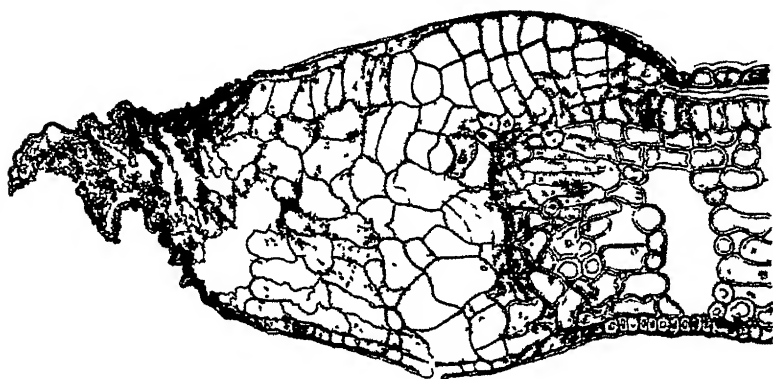


FIG. 7.—Transverse section through younger leaf of *Beberis nervosa* wounded before maturity, pseudocicatrice very compact and cicatrice involving massive development of wound cork which in some cases ruptures epidermis; normal leaf tissue at right of figure; killed 40 days after wounding

erable width of tissue the air spaces are occluded. There is no suggestion of wound cork in these leaves under such circumstances.

WOUND RESPONSES OF YOUNGER LEAVES

Among the leaves of *B. nervosa*, wounded and collected for the writer by Dr. GEORGE B. RIGG of the University of Washington, was one with well developed cicatrice along all wounded margins (fig. 7). Recalling that the wound responses of older and younger leaves may vary considerably, the writer assumed, and apparently correctly, that the leaf thus forming a cicatrice had been wounded earlier in its development. The following year Dr. RIGG kindly carried out further wounding experiments and intentionally included younger leaves which had developed that spring. Among these, when pre-

served and shipped to our laboratory, were found many that had formed the pseudocicatrice, and this in turn had been followed by a cicatrice sometimes of striking development.

In fig. 7 are shown the wound responses of an immature leaf wounded on May 21 and killed June 30, 1927. It may be noted that there had been complete collapse of the mesophyll following lesion. Not only had the interior tissues crumpled, but both epidermal layers were drawn sharply inward, the leaf shrinking to less than one-third its original thickness and giving rise in this way to a most efficient pseudocicatrice. It was significant to find that under such circumstances a cicatrice also was developed, and that wound cork was formed in abundance. In most instances the cicatrice region was swollen to nearly double the normal leaf thickness, and in some cases the growth of wound cork even burst the sturdy epidermis on the under side of the leaf. All cell layers contributed to this riot of wound tissue development, and the heavy subdermal layer was displaced through a considerable distance (fig. 7).

A study of leaves of *Berberis nervosa* wounded at varying ages shows a close correlation between the degree of pseudocicatrice development and the amount of cicatrice formation. Intermediate stages, in which the dead leaf tissues along the wound margins collapse to about one-half the normal leaf thickness, show considerable activity of living cells along the upper part of the leaf. In such cases, behind the partial pseudocicatrice, mitoses in the palisade cells produce two, three, or occasionally four layers of cork extending through a considerable distance along the upper epidermis, and resulting in a decided bulge on this surface. The lower portion of the leaf, however, responds by the formation of tyloses, and these are restricted to a relatively narrow zone. Fungal mycelia are but sparingly developed in such cases.

In those instances where the leaves were younger and dead tissues collapsed in greater degree, approaching the normal pseudocicatrice, cork formation involved all layers of the leaf, and thus provided a symmetrical healing tissue of wound cork launched evidently through mitoses in all cell layers. In this species there is therefore a diminishing capacity for wound response as the leaf matures, and all intermediate stages may be secured between the extremes shown in figs. 4 and 7.

Practically every section through any leaf of *Berberis nervosa*, if mature when wounded, shows numerous fungal hyphae and spores in the dead marginal tissue. The most conspicuous fungus is a species of *Fumago*, as determined by Professor G. W. MARTIN, and the sclerotia of this form may literally blacken the wounded leaf margin (figs. 4-6). The growth of this particular fungus may have been favored directly or indirectly by stored starch left in the dead cells, which in the absence of a pseudocicatrice, were freely exposed to fungal invasion. No such growths were noted in the leaves of this species if wounded at an earlier stage when both cicatrice and pseudocicatrice could be developed (fig. 7).

Mycelia of fungi have been noted rarely in studying the wounded leaves. Among some hundreds of species examined, many of them natural wounds, the only other ones regularly harboring living organisms were certain thick-leaved New Zealand evergreens which had yeastlike cells in the pseudocicatrice. Lodged spores have often been noted, but active mycelia seem rare. This is in harmony with the general observation that exposed foliage leaves, although subjected to constant injury, are seldom infected through open wounds. Even spores carried on the biting parts of insects may be excluded by the barriers subsequently developed, for the dying and drying of exposed leaf tissue, as the pseudocicatrice forms, may inhibit spore germination or mycelial growth. Meanwhile the whole marginal region is soon walled off by the tissues of the cicatrice proper. Of course when the spore or inoculum is introduced into the tissues by a suctorial insect such protective devices do not function. Following lesions of this type, neither pseudocicatrice nor cicatrice is formed, and infection if it occurs is underneath the normal leaf coverings.

Discussion

The broad-leaved evergreens discussed in this paper offer numerous contrasts to the mesophytic types previously described (3). Their leaves are more massive than those of the deciduous forms, cuticular coverings are much heavier, and all cell walls are thicker, especially in *Berberis nervosa*. In addition to these structural qualities there are probably other differences, doubtless of no less significance, relating to the content and modified activities of the cells. Wound responses, however, with the exception of *B. nervosa*, are

similar in a general way to those exhibited by mesophytic foliage, although the process is much slower and wound cork is usually produced much more abundantly.

These generalizations are not based alone upon the three species discussed in this paper. Unpublished data relate also to the broad-leaved evergreens from southern California, the strand vegetation of the Fiji Islands, and the preponderantly evergreen flora of North Island, New Zealand. In the considerable number of species examined from these widely separated regions, lesion of exposed leaves regularly results in the establishment of a pseudocicatrice through the collapse of dead tissues. This in turn is followed by the development of a more or less extensive cicatrice through the activity of living cells.

Recalling the more massive structure and heavier cell walls of such leaves, many of them sclerophylls, one might not have anticipated the regular formation of this preliminary barrier. While its initiation begins promptly, the period through which drying and dying back continues may be considerably extended, owing to differences in cell structure and content. The pseudocicatrice finally produced, however, is usually much more extensive than in the thinner and softer leaves; and the heavy cuticular layers, which are commonly involved, add much to its efficiency.

Comparable differences are noted in the cicatrice proper. Its beginning in these leaves is considerably delayed, and complete development requires much longer than in mesophytic leaves. The initial mitoses may not take place until ten days after lesion, and complete development requires a month or more. While the cicatrice is commonly more massive than in the mesophytes, owing to its tardy development there is a much longer period of dependence upon the pseudocicatrice for protection against traumatic water loss and possible infection.

The single exception encountered in this general survey of evergreen angiosperms was *Berberis nervosa*. With walls of its mature leaves too strong to collapse following the death of protoplasts, no pseudocicatrice can result; unique also is this form in its inability to develop wound cork under these conditions, and doubtless the common presence of fungi in the wounded margins may be correlated

with the lack of the usual protective barriers. Of no less interest is the strikingly different behavior of the younger leaves of this species. If wounded before the cell walls have fully thickened, an efficient pseudocicatrice is followed by an unusual development of wound cork. Leaves wounded at varying stages of maturity show different degrees of tissue collapse, and there is likewise a close correlation between the amount of pseudocicatrice development and that of wound cork formation. In fact all types of response might be brought about by wounding the leaves of a single plant at different stages of foliar development. It probably is not accidental that the growth of fungi in the wounded margin is inversely proportional to the development of these barriers.

It is unfortunate that a mature foliage leaf cannot regenerate the epidermal and cuticular layers when wounded. A cuticle less than $1\ \mu$ in thickness seems to meet admirably the problems of the average leaf's protection, but if wounded, the traumatic responses usually involve a wide zone 50–150 cells in depth, in healing the lesion. While very young leaves are reported to regenerate the epidermis when injured, older ones seldom do this even indirectly. Apart from the greater plasticity of younger leaves, the older foliage organs are necessarily in exposed position, and marginal cells abruptly laid bare by wounds cannot meet the problems of excessive water loss and maintain turgor long enough to develop a cuticular covering.

The cicatrice formation of a leaf is in principle like the development of healing tissues in stems, and especially in tubers. There is the establishment of a preliminary phellogen layer, and the subsequent development, by means of its activities, of a mass of wound cork. All of this takes considerable time, and in the case of leaves, gives rise to a healing tissue actually far in excess of the protective needs of the organ. The cicatrice tissues developed by a wounded leaf are therefore not peculiarly foliar specializations, but are typical of massive plant organs. On the other hand, the pseudocicatrice is distinctively a foliar response. Its favorable development is contingent upon cell structure, tissue arrangement, and organ form not usually found in stems or other massive plant parts. The fortunate combination in foliage leaves of a thin, flattened form, with soft interior mesophyll having yielding epidermal layers covered by a thin

elastic cuticle, provides for ready collapse of cells exposed by wounds, and results in a marginal protective barrier produced by these dead tissues. Perhaps without this pseudocicatrice the highly specialized foliage leaf, subject to constant injury, would have been impossible.

It remains for experimental work to interpret the causal relations between pseudocicatrice and cicatrice proper; there is considerable evidence that the latter is not only sequential to the other in time, but is likewise dependent upon it for development. Apart from such considerations, however, the pseudocicatrice is of primary importance as a protective device, and plays an even more important part in broad-leaved evergreens than in mesophytes.

Summary

1. Leaves, as necessarily exposed organs, do not employ massive coverings, and so are constantly subjected to injury.

2. Following lesion, tissue collapse along wounded leaf margins usually leads to the prompt establishment of a protective barrier, the pseudocicatrice.

3. The cicatrice proper of wound cork forms slowly underneath, from living tissues protected by the pseudocicatrice.

4. The broad-leaved evergreens in comparison with mesophytic foliage have thicker leaves, heavier cell walls, and better developed cuticular layers. The wound responses are similar, but the process is slower in the thicker leaves and their protective structures involve much greater amounts of tissue.

5. The mature leaves of *Berberis nervosa* are unique because they are so rigid that the exposed tissues cannot collapse; consequently these leaves are unable to form a pseudocicatrice, and this in turn inhibits the development of any cicatrice. Such leaves are left open to fungal invasion and usually have masses of spores and mycelia in and along the wounded margins.

6. If younger leaves of the same plants are wounded, the dead tissues collapse into an efficient pseudocicatrice, while behind this is formed a massive cicatrice of wound cork, and such protected margins have no fungi. These leaves reveal a diminishing capacity for the formation of such protective barriers as they approach maturity, and show increasing amounts of fungi along their wounded margins.

7. The cicatrice proper is generally not initiated until several days after injury, and its complete development in broad-leaved evergreens requires 20-40 days. While highly efficient, it is usually more elaborate than necessary, and corresponds in many respects with the healing tissues of tubers or other massive plant parts.

8. The pseudocicatrice is distinctively a foliar response. The structure and arrangement of cells in the leaf in combination with its thin and flattened form permit the ready collapse of tissues exposed by wounds, with favorable use of the cuticular layers. The pseudocicatrice, in addition to its protective value, sustains a causal relation to the development of wound cork and remains a permanent barrier along the wounded leaf margin.

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[Accepted for publication April 14, 1931]

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RELATIVE ABUNDANCE OF STOMATA IN CITRUS AND SOME RELATED GENERA¹

E. HIRANO²

(WITH ONE FIGURE)

Citrus trees are cultivated in tropical and subtropical regions under a variety of climatic and soil conditions. In semiarid regions there are often deficits of water which induce a suspension or temporary modification of fundamental physiological processes. Fluctuations in the water content of citrus leaves were reported by CORR and HODGSON (3), who pointed out the effects of the deficits upon the abscission of young fruits. BARTHOLOMEW (2) described the effects of daily deficits of water in the lemon tree, and REED and BARTHOLOMEW (6) studied the effects on orange trees of more prolonged water deficits. OPPENHEIM (5) described the diurnal changes in the activity of stomata and made determinations on their density in *Citrus aurantium* and *C. aurantifolia*.

The writer studied the relative abundance of stomata in the leaves of various species of *Citrus* and related genera, with special reference to their geographical origin, so far as it could be ascertained. The material for this study was collected in a restricted area, however, namely, the orchard of the Citrus Experiment Station at Riverside, California. The study may therefore be expected to indicate whether there were any heritable differences in the abundance of stomata when all the species and varieties were grown simultaneously under the same climatic and geographic conditions.

I am indebted to Dr. H. J. WEBBER for freedom to use the necessary material, and to Dr. H. S. REED for the facilities of the laboratory of plant physiology, as well as for his advice and assistance.

The leaves used for this study were collected during a period ex-

¹ Paper no. 244, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² Professor HIRANO spent most of the year 1929 as a guest investigator in the Citrus Experiment Station. This manuscript contains some of the results which he obtained during that time (H. S. R.).

tending from September 16 to 30, 1929, from shoots produced during the first cycle of growth of that season. Care was taken to select full-grown leaves from non-fruiting shoots which grew in full sunlight on the south side of the tree. For each species or variety a sample of 15 to 20 leaves of similar area from as many shoots was selected. Particular attention was paid to the selection of leaves of approximately the same size and age, since it has been found that these factors influence the density of stomata (REED and HIRANO 7).

TABLE I

CLIMATOLOGICAL DATA FOR 1929 AT RIVERSIDE, CALIFORNIA; LATITUDE 34° N.;
ELEVATION ABOVE SEA LEVEL, 851 FEET (262 M.)

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Precipitation (mm.).	35 3	21 1	23.4	64 5	Trace	Trace	0	0	16 0	0	0	0
Mean temperature (° F.)	49 6	49 2	54 8	56.2	65 2	69 7	76 2	79 0	70 8	67 0	58.4	60.6
Sunshine*												
Actual number of hours.	209	236	275	259	300	350	352	374	265	283	299	246
Per cent of possi- ble	66	77	74	66	69	81	80	90	71	81	96	80
Relative humidity (percentage).	79	67	66	73	68	60	65	53	80	61	42	63
Evaporation from pan (inches)	2 03	20 53	25 4	92	6 0	7 86	11 39	8 77	7 23	4 93	5 56	3.97

* Data on sunshine taken from records obtained by Weather Bureau in Los Angeles

After measuring the leaf areas with a planimeter, 10 similar leaves were selected from each species or variety and small pieces of tissue cut from the middle of each leaf. These pieces were kept in acetic-formalin-alcohol until the stomata were counted.

The number of stomata in 20 areas of 0.29 sq. mm. was determined for each leaf, and from this determination the average number per square millimeter was computed. The number of stomata in 200 distinct areas for each species or variety employed was therefore determined. Areas in the vicinity of large veins, oil glands, and trichomes were avoided, since they contain relatively few stomata.

The nature of the climatic conditions prevailing at Riverside may be inferred from the brief summary given in table I. The data show that the rain of the year fell principally in the first four months, and that evaporation was high in the seven months following April. To

be more exact, more than half the precipitation occurred in the months of March and April, a period in which many of the new leaves were growing. The importance of temperature and precipitation in the months of March, April, and May is indicated by data obtained by BAHGAT (1) and summarized in table II. It is impossible, of course, to dissociate the effects of the two factors in the present case, but it appears probable that the abundance of stomata shows a closer correlation with precipitation than with temperature.

BAHGAT (1) found that (1) density of stomata on the leaves of a given species or variety of *Citrus* was greatest in hot arid regions

TABLE II

RELATION OF NUMBER OF STOMATA TO AMOUNT OF PRECIPITATION AND TEMPERATURE IN VARIOUS LOCALITIES IN CALIFORNIA (DATA ON STOMATA TAKEN FROM BAHGAT)

LOCALITY	LATITUDE (N.)	MEAN TEMPERATURE		PRECIPITATION		NO STOMATA PER SQ. MM						
		March to June (° F.)	Year of 1922 (° F.)	March to June (mm.)	Year of 1922 (mm.)	Washington Navel	Sour orange	Grapefruit	Eureka lemon	Valencia	Mandarin	Dancy tangerine
El Centro*...	32°51'	73 0	71 0	Trace	65 3	550	498	617	555	465		
Riverside ..	34° 0'	62 8	62 8	77 2	343 9	468	434	479	530	465	420	466
Porterville.	36° 0'	65 8	64 4	104 1	320 9	348	390	463	494	372	382	390
Berkeley. . .	37°54'	56 5	56.0	111 3	764 54	358	364	399	480		363	376

* Data recorded at Calexico near El Centro.

and least in cool moist regions; (2) shading the leaves reduced the density of stomata and increased their size; (3) leaves of the grapefruit and lemon had a greater density per unit area than other varieties studied, while a smaller number was found on leaves of the kumquat and trifoliate orange.

In presenting the following report, the precipitation for the spring months (March 1 to June 1) is recorded in addition to the amount of annual precipitation. When dealing with places in the southern hemisphere, data for the corresponding season (September 1 to December 1) are used. Most of the data on rainfall in tables III and IV were obtained from standard works on meteorology, among which FISCHER (4) should be noted.

The ecological relations of cultivated citrus trees are difficult to define, because the cultivator employs irrigation of the soil to avoid

TABLE III

DENSITY OF STOMATA ON LEAVES OF VARIOUS SPECIES OF CITRUS GROWN
AT RIVERSIDE, CALIFORNIA, IN 1929

C E S RECORD NO	SPECIES OR VARIETIES	AVER- AGE LEAF AREA (SQ. CM.)	AVERAGE NO. STO- MATA PER SQ. MM.	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITATION		LOCATION OF STATION
					Spring mos. (mm.)	Annual (mm.)	
628	C. aurantium Standard Seed- ling	36.0	414 ± 2.3	Western Spain, domesticated in Florida	87 495 431	438 1525 1374	Seville, Spain Miami, Fla. Bartow, Fla.
1110	Brazilian.	22.9	440 ± 2.5	Brazil	225 481 382	787 1910 1091	Caetite, Bahia, Brazil So. Bento das Lag- er, Bahia, Brazil Rio de Janeiro, Brazil
660	Paraguayan	21.7	459 ± 2.7	Wild in Paraguay	380 509	1423 1465	Asuncion, Paraguay San Salvador, Para- guay
1226	Chinese "Tsen Tze"	40.2	465 ± 3.0	Ichang, China; wild in Himalaya	428 284 140	1059 1024 955	Ichang, China Yunnanfou, China Allahabad, Punjab
64	South African	23.6	490 ± 2.1	So. Africa	445 175 322	1055 550 746	Durban, Natal Bloemfontein, Orange Free State Pretoria, Trans- vaal
564	C. aurantifolia Bears Seedless lime	13.2	326 ± 2.4	Originated in Cali- fornia	118	440	Los Angeles, Calif.
391	Tahiti lime	14.2	375 ± 3.2	Tahiti Island	481 431	1410 1373	Papeete, Tahiti Bartow
452	Kusai lime	14.5	595 ± 2.8	Kusai Island, im- portant lime for Hawaii (Hume)	632 210 665	1760 755 2100	Lalabafen, Kusiae Honolulu Av. of all Hawaiian Island
1710	Mexican lime..	13.6	514 ± 2.6	Hawaii; wild in for- est of So. Florida	210 495	755 1524	Honolulu Miami
1214	C. cambroviorexa (probably C. hystrix)	10.6	384 ± 2.9	Tahiti	482	1410	Papeete
1665-B	C. junos Yuzu	12.8	429 ± 2.3	Wild in Yunnan; cultivated in Ja- pan, Korea	281 555	1024 1476	Yunnanfou Tokyo, Japan*
1216	Kansu f.	12.2	402 ± 2.4	Hupei, China; cul- tivated in Japan	555	1476	Tokyo*
432	C. hystrix Large leaves and large smooth fruit.	28.0	550 ± 2.4	Islands of tropical Pacific, Indian oceans	381 871	1962 2190	Manila, P.I. Colombo, Ceylon
	Small leaves and small corru- gated fruits.	12.8	704 ± 3.5

TABLE III—Continued

C. E. S. RECORD NO.	SPECIES OR VARIETIES	AVER- AGE LEAF AREA (SQ. CM.)	AVERAGE NO. STO- MATA PER SQ. MM.	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITA- TION		LOCATION OF STATION
					Spring mos (mm.)	Annu- al (mm.)	
400	<i>C. limonia</i> Rough lemon ..	39.3	552 ± 2.8	Naturalized in for- ests of So. Fla.	{ 495 431	1525 1374	Miami Bartow
1482	Sweet lemon. .	39.1	635 ± 2.5	Cultivated in Medi- terranean regions	{ 137 116	590 362	Palermo, Italy Murcia, Spain
	Eureka lemon	37.0	636 ± 2.5	Originated in Los Angeles	118	400	Los Angeles
584	Lisbon lemon .	40.5	743 ± 3.6	Mediterranean and California	{ 116 137 118	362 590 400	Murcia Palermo Los Angeles
	<i>C. maxima</i>				{ 427	1794	Union de Reyes, Cuba
1462	Cubanshaddock	44.8	476 ± 3.7	Cuba	{ 507	1396	Average of 19 ob- servatories in Cuba
1852	<i>C. nobilis</i> Kunembo ..	37.6	377 ± 4.8	Liu-kiu Island, Jap- an	{ 337	1092	Havana, Cuba
277	Dancy ..	12.1	415 ± 1.8	Florida and Foo- chow, China	{ ? 431	1515 1374	Foochow, China Bartow
	Unshiu (Satsu- ma) ..	40.6	446 ± 2.8	Southern Japan, Florida, Ala- bama, etc.	{ 431 532	1374 1476	Bartow Tokyo
594	King.	13.3	463 ± 2.7	Cochin-China, Flo- rida, China, Jap- an and Luchu	431	1374	Bartow
270	Cleopatra....	11.8	552 ± 3.7	Florida and Algiers	{ 431 196	1374 546	Bartow Constantine, Al- giers
696	Kinokuni	12.7	582 ± 2.6	Kiangsi and Che- kiang; imported from Ichang, China	428	1059	Ichang
325	<i>C. paradisi</i> Chinese pomelo	42.9	532 ± 3.8	{ 431 495	1374 1525	Bartow Miami
577	Shaddock (Cal- cutta)	40.8	571 ± 4.0	Calcutta	534	1652	Calcutta
454	Shaddock (Ha- waii).....	40.7	626 ± 3.0	Honolulu	211	756	Honolulu
579	Ditto.....	44.3	630 ± 3.1	Vicinity of Kilauea	211	756	Honolulu
138-B	<i>C. medica</i> Indian citron...	21.1	552 ± 2.2	Seeds from India	534	1652	Calcutta
757	Phillipine citron	32.8	623 ± 3.3	Seeds from Lamao Exp. Sta. Batan, P.I.	382	1962	Manila
601	Chinese lemon.	22.5	643 ± 3.3	Mongtze, Yunnan- fou, China	317	955	Mongtze, China
128	Italian citron.	19.6	873 ± 4.7	Buds from Posilipo	137	590	Palermo

TABLE III—*Continued*

C E S RECORD NO	SPECIES OR VARIETIES	AVER- AGE LEAF AREA (SQ. CM.)	AVERAGE NO STO- MATA PER SQ MM	NAT'L PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITA- TION		LOCATION OF STATION
					Spring mos (mm)	Annua- l (mm)	
1458	C. mitis	13.4	665 ± 7.7	Endemic in Philip- pines and interior of China	382	1903	Manila
	Marsh	42.4	583 ± 3.2	West Indies, culti- vated in Florida and California	117 131 495	400 1371 1525	Los Angeles Bartow Miami
448	Round Hawai- ian pomelo	41.0	584 ± 2.6	Honolulu	210	756	Honolulu
255	C. sinensis Lue Gim Gong orange	40.8	402 ± 2.5	Florida	431 495	1373 1525	Bartow Miami
	Washington Navel orange	41.1	458 ± 2.7	Originated in Bra- zil, cultivated mainly in Cali- fornia, So Ameri- ca, Australia, So Africa	225 117 322	787 400 746	Cactiti, Bahia Los Angeles Pretoria
282	Jaffa orange	38.8	473 ± 1.8	Mediterranean	266	947	Beirut, Syria
	Valencia orange (large leaf)	38.7	504 ± 2.7	California and Mediterranean	113 117	476 400	Valencia Los Angeles
591	St. Michael orange (paper rind)	41.2	531 ± 2.7	Mediterranean	107 130 167	608 430 976	Malta Alicante, Spain San Miguel, Azores
299	Ruby blood orange	36.1	533 ± 2.5	Mediterranean	183 113	771 476	Philippeville, Al- giers Valencia
1455	C. webberi	9.5	769 ± 6.0	Endemic in Philip- pines	382	1963	Manila

* Precipitation at Tokyo is not representative of precipitation in Japanese orange regions, but since data for orange regions is not at hand, the precipitation of Tokyo is used here.

† Relationships of Kansu not yet determined

drought, heating devices to maintain temperatures above the freezing point, and other means for making a suitable environment. Nevertheless cultivators have found by the trial and error method that certain varieties may be grown successfully in certain localities but not in others.

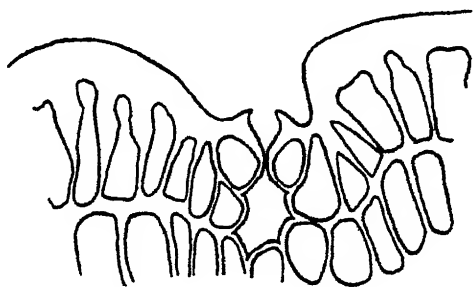
It seems evident from the data in tables III and IV that species whose leaves have an average density of 500 or more stomata per square millimeter thrive in the tropics, with the exception of lemon

TABLE IV

DENSITY OF STOMATA ON LEAVES OF VARIOUS SPECIES OF AURANTIOIDEAE
GROWN AT RIVERSIDE, CALIFORNIA, IN 1929

C. E. S. RECORD NO.	SPECIES OR VARIETIES	AVER- AGE LEAF AREA (60 CM.)	AVERAGE NO. STO- MATA PER SQ. MM. (20 COUNT- 10 LEAF)	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITA- TION		LOCATION OF STATION
					Spring mos (mm.)	Annu- al (mm.)	
1465	Eremocitrus glauca	0.6	167 ± 1.7	Desert in north-eastern Australia	?	?	.
1484	Microcitrus australasica	0.4	407 ± 2.5	Moreton Bay, near Brisbane, Queensland	301	1265	Sydney, Australia
1466	Faustriamedia (C. mutis x M. australasica)	1.6	409 ± 2.0
1468	Fortunella margarita	8.6	461 ± 3.3	Japan	552	1476	Tokyo, Japan
1484	Microcitrus australasica var sanguinea	0.4	485 ± 2.2	Tamborine Mountain, So. Queensland	301	1265	Sydney
	Poncirus trifoliata	5.0	495 ± 3.8	Western Hupeh, Ichang, Shensi, China, Japan and Korea	428 552	1059 1476	Ichang, China Tokyo
1637	Chalcas exotica	10.6	501 ± 2.2	Indian peninsula, India, Ceylon, Burma, Indo-China, Malay, China, Japan, etc.	534 871	1652 2190	Calcutta, India Colombo, Ceylon
1433	Balsamocitrus paniculata	6.7	543 ± 2.7	Tropical W. Africa (Nigeria, Gold Coast)	.. .	2000-3000	Nigeria; most parts of Gold Coast
1432	B. gabonensis	12.2	553 ± 2.3	Pahouin No. French Congo, So. Kamerun	..	2000-3000	Kamerun-No. French Congo
1450	Citropsis schweinfurthii	10.4	589 ± 3.5	Uganda, Congo, Sudan	..	2000-3000	Congo, Uganda, most parts of Sudan
1471	Chaetospermum glutinosum	8.6	592 ± 3.0	Central part of Luzon	382	1963	Manila, Luzon, P.I.
1430	Atalantia citroides	9.1	697 ± 2.5	Cochin-China, Cambodia	1600 1755	1956 2159	Saigon, Cochin-China, Khong, Cambodia
1638	Atalantia disticha (blanco)	2.9	867 ± 4.8	Philippines	382	1963	Manila, Luzon, P.I.
				So. China, Hongkong, Kwantung, Hainan, Tonkin	327 548	2795 1614	Hue, Annam, Tonkin
1492	Severinia buxifolia	3.5	1064 ± 3.7	Hainan, Tonkin, Annam, Formosa, etc.	526 737	1631 2005	Hoihow, Hainan, China, Hongkong, China

and grapefruit. It should be noted, however, that the lemon is probably a native of India, although it is extensively cultivated in Sicily and California. Species which have a stomatal density less than 500 are generally found in regions between 23.5 and 44 degrees of latitude north or south of the equator, with the exception of *Citrus aurantifolia* and *C. cambodioxora*. A variability of less than 100 stomata per sq. mm. was observed among the varieties of *C. aurantium*, *C. junos*, and *C. paradisi*; of 100-200 stomata per sq. mm. in *C. hystrix*, *C. limonia*, *C. maxima*, and *C. sinensis*; while a variability of more than 200 stomata was found among varieties of *C. aurantifolia*, *C. medica*, and *C. nobilis*. The abundance of stomata in the leaves of certain other genera of Aurantioidae was also investigated, so far as material was available in the orchard of the Citrus



0.01 mm.

FIG. 1.—*Eremocitrus glauca*, section through stoma.

Experiment Station. The results (table IV) will be presented before proceeding to discuss the more general problem.

The species listed in table IV are not cultivated to any great extent, and it is therefore interesting to compare their characters, because it may be assumed that they have been but little changed by artificial selection or by long cultivation outside their habitats. The density per square millimeter varied from 167 in the leaves of *Eremocitrus glauca* to 1064 in the leaves of *Severinia buxifolia*. *E. glauca* is a native of the Australian deserts, and has characters like those commonly found in desert plants, small pubescent leaves, stomata imbedded in the body of the leaf (fig. 1), etc. The number of stomata per square mm. was very small. *Microcitrus australasica* and its variety *sanguinea* also have very small leaflets, but the density of stomata is much greater than that of *E. glauca*. The leaves of *Faustrimedin*, a hybrid, resemble more closely in the matter of size and density of stomata those of *M. australasica* than any other parent.

It will be noted that the species which had a stomatal density less than 500 occur generally in subtropical and temperate zones, and that species having a density greater than 500 occur generally in the tropical zone, although *S. buxifolia* and *C. exotica* have a wide range and extend somewhat into the subtropics. The number of species observed is not large, and one must be cautious about drawing rigid conclusions from them. However, they indicate strongly that the species and varieties of tropical regions studied have a greater stomatal density than those of extra-tropical regions. I hope to study this problem further. The two species of *Atalantia* had a relatively high stomatal density, but *Severinia buxifolia* was considerably higher than any other species studied.

In considering the relative abundance of stomata in the species and varieties listed in tables III and IV, the discussion will deal incidentally with the potentiality of the environmental factors which affect their abundance on the leaves of *Citrus* and related plants. The number of stomata per unit area in various plants has been found by previous investigators to be influenced by light intensity, water content of soil, and atmospheric humidity. The effect of air temperature has not been studied sufficiently to warrant any positive statements about it. High temperatures are usually associated with strong light intensity and low humidity. Table II indicates that precipitation during the period in which leaves are developing is more important than temperature. For example, the differences between the numbers of stomata in various species at Porterville and Berkeley are not significant, but the differences in precipitation in the spring months is likewise small.

The intensity of insolation in the tropics is greater than in other regions of the earth, and this is associated with a higher mean temperature. Both insolation and temperature may be reduced locally by precipitation and attendant cloudiness, although the mean cloudiness in various latitudes does not vary as much as might be expected. The ensemble of the factors and not individual factors needs to be borne in mind when considering the generic and specific differences in leaf size and stomatal density presented in tables III and IV.

Between latitudes 23.5° N. and 40° N. there are two distinct cli-

matic conditions with respect to citrus culture; namely, Mediterranean climate and Temperate climate. The Mediterranean climate mostly predominates in the citrus districts along the Mediterranean Sea and the southwestern part of the United States (southern California and Arizona). In these districts the annual precipitation is small, and most of the rainfall comes in winter as intermittent storms separated by spells of fine sunny weather. Although the natural intensity of light in these regions may be inferior to that of the tropics, therefore, the absorption of light by clouds, rains, etc., is low and the moisture in air and soil is very small. Such conditions may be more favorable for the development of high stomatal density, or for the adaptation of *Citrus* varieties having high density, than other districts of similar latitude having moist climates. These districts contain many famous commercial orchards including varieties of comparatively high density; that is, various kinds of lemons, sweet oranges, and grapefruits. Even in these districts the distribution of species is governed by slight changes in climatological factors. The general classification of varieties of *Citrus* of subtropical regions of southwestern United States having the so-called Mediterranean climate will give some idea of the relations between stomatal density and adaptability to climatic conditions (table V).

The Navel orange, which has the lowest density of stomata among the typical varieties, is grown in the northern part of this area, which is characterized by light to moderate rainfall, hot dry summers, and cold winters. The Valencia orange has a slightly higher density of stomata than the Navel orange, and is grown somewhat farther south and nearer the sea coast, where there is rather more rainfall in the winter and higher humidity in the summer. The grapefruit, which has a higher density of stomata than the Valencia orange, is grown in the more arid regions of the southwest. It seems that the climatic factors associated with precipitation and intensity of light have an important influence on the geographical distribution of these three varieties.

The citrus districts having the so-called Temperate climate appear to have an annual precipitation which is a little less than that of the tropics. Although precipitation is usually much greater in the summer growing season than in the winter resting season, there are

no such distinct seasons of extreme dryness and wetness as in the so-called Mediterranean climate or in the tropics.

The main citrus districts in the Temperate climate seem to be poorly suited to the culture of the lemon tree, but are well adapted

TABLE V

GENERAL CLASSIFICATION BY REGIONS OF SOUTHWESTERN U. S. WITH TYPICAL CITRUS VARIETIES AND DENSITY OF STOMATA

REGIONS	LOCALITIES	CLIMATICAL CONDITIONS	APPROXIMATE LATITUDE (N)	TYPICAL CITRUS VARIETIES	STOMATA PER SQ. MM.
Desert regions (California and Arizona)	Imperial, Coachella, Salt River and Colorado River valleys	Intense summer heat, practically no rains	32°-33° 40'	Grapefruit (Marsh)	583
Great Interior Valley of California	Sacramento, San Joaquin	Hot and dry in summer, cold in winter; light to moderate rainfall	35°30'-40°30'	Navel orange	458
Southern Interior valleys of California	Riverside, Redlands, San Bernardino, Pomona	Moderately hot and dry in summer, some coastal influence in winter; medium rainfall	34°	Navel orange	458
				Valencia orange	504
Southern coastal region of California	San Diego, Orange, Los Angeles, and Santa Barbara counties	Cool in summer, rarely cold in winter; humidity usually high; light rainfall	32°30'-35°	Lemons (Eureka and Lisbon)	636-743
				Valencia orange	504

to the culture of grapefruit (central Florida), sweet oranges (Florida, southeastern China, Rhodesia, and Brazil), Satsuma (Japan), and shaddocks (Amoy). The cooler parts of the Temperate climate are those in which varieties of low stomatal density are cultivated, for example, Yuzu, Kansu, and Kunembo (Japan). The citrus districts of California and Japan which lie in the same latitude are marked by differences in their adaptability to varieties, which may

be due to the fact that one has a Mediterranean and the other a Temperate type of climatic factors.

The distribution of varieties of a single species also seems to be correlated with stomatal density. For example, four shaddocks (table III) are grown in the latitude between 20° N. and 21° N. The shaddock from Honolulu and the wild shaddock in the vicinity of the volcano Kilauea, near Honolulu, have higher density of stomata than those from Calcutta and Cuba, where there is much heavier precipitation. Three of the citron varieties, Indian citron, *C. medica* from the Philippine Islands, and Chinese lemon, are grown in the tropical zone. Precipitation during the spring months is higher in India than in China and the Philippine Islands, and the stomatal density appears to correspond to these differences in precipitation. The Italian citron, which has the highest density among the citrons, came from the typical Mediterranean climate.

In the *C. nobilis* (mandarin) group, Kunembo, Unshiu (Satsuma), Dancy, and King, which are low in stomatal density, thrive in the regions of spring and summer rains such as the northern coast of the Gulf of Mexico and southern Japan. It will be noted that the spring rainfall at Bartow, Florida, is greater than the total annual rainfall at Los Angeles. The orange varieties which have higher stomatal density, such as Ruby blood, St. Michael, Valencia, Jaffa, Navel, etc., seem to thrive in the localities where the climate is comparatively tropical (Rhodesia, Brazil, Canton, etc.), or in the dry so-called Mediterranean climate (coast of Mediterranean and southern California).

In connection with the relation of hardiness and stomatal density, it seems apparent that hardiness depends entirely upon the condition of the cell content, although there seems to be some coincidence of hardiness with density of stomata of the commercial species. Data given in table VI show some relation between stomatal density at Riverside, the latitude in which the variety or species is cultivated, and the hardiness of the tree with respect to low temperatures. With the exception of the lime (*C. aurantifolia*), the hardiness seems to be associated with low density of stomata. This relationship might be anticipated from the fact already mentioned, that species predominating in tropical regions of lower latitudes have a high

TABLE VI

RELATIVE DENSITY OF STOMATA IN RELATION TO HARDINESS TO COLD AND TO
LATITUDE OF PRINCIPAL LOCALITY OF CULTIVATION

SPECIES	NO OF VARIETIES STUDIED	AVERAGE STOMATA PER SQ MM	HARDINESS	LATITUDE (° N)	MAIN LOCALITY
<i>C. webberi</i>	1	769		9-20°	Philippines
<i>C. medica</i>	4	673	Tenderest of all cit- rus	5-15°	Malay peninsula; Malabar coast
<i>C. mitis</i>	1	605		9-20°	Philippines
<i>C. limonia</i>	4	642	Somewhat tender	{ 0-15° 37-38° 32-36°	Malay peninsula Sicily California
<i>C. hystrix</i>	2	627	Tender	0-23°	Tropical Pacific Island, Ceylon
<i>C. maxima</i>	4	576	Moderately hardy	{ 13-20° 24-27°	Bangkok, Siam Amoy, China
<i>C. paradisi</i>	3	566	"	18-30°	Cultivated in West Indies and Florida
<i>C. sinensis</i>	6	484	"	{ 23 16-23°* 26-30° 32-36° 37-44°	Canton Brazil Florida California Mediterranean re- gion
<i>C. nobilis</i>	6	473	Moderately to very hardy; Satsuma is the hardest of all edible oranges	King group. 28-30° Tangerine group: { 23° 26-30°	Chekiang, China; Florida Fuchow, China Florida
<i>C. aurantium</i>	5	454	Moderately hardy	Satsuma group: { 28-32° 31-35° 26-30° 37°	Florida to Alabama Japan Florida Seville, Spain

* S latitude.

TABLE VI—*Continued*

SPECIES	NO OF VARI- ETIES STUDIED	AVERAGE STOMATA PER SQ MM	HARDINESS	LATITUDE	MAIN LOCALITY
<i>C. aurantifolia</i>	4	453	Moderately tender intermediate be- tween citron and lemon	0-23 9-20°	Malay to Canton Philippines
<i>C. junos</i>	2	416	Very hardy	31-40°	Japan
<i>Fortunella mar- garita</i>	1	461	"	31-40°	"
<i>Poncirus trifoli- ata</i>	1	495	"	31-40°	"

stomatal density, while those growing in the moist regions of higher latitudes have a low stomatal density.

Summary

1. Precipitation of rain during the spring months, while the young citrus leaves are developing, showed a stronger correlation with the number of stomata than the amount of annual rainfall; although it is impossible to disregard other factors, such as light, heat, and atmospheric humidity.

2. Species and varieties of the Aurantioideae show differences in the density of stomata which appear related to their place of origin. For example, most species growing in the tropics have more than 500 stomata per square millimeter, while those outside the tropics have a lower density.

3. Certain species, like *C. paradisi*, show adaptability to a wide range of conditions, growing both in the hottest districts and in the moist temperate region. Other species, like *C. limonia*, grow most successfully in regions having the Mediterranean type of climate.

4. The hardier varieties and species of citrus trees are characterized by low stomatal density, although there are a few well marked exceptions.

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[Accepted for publication April 21, 1931]

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SOME CARYOPHYLLACEOUS PLANTS INFLUENCED IN GROWTH AND STRUCTURE BY ARTIFICIAL ILLU- MINATION SUPPLEMENTAL TO DAYLIGHT

FRANCIS RIVALEX

(WITH EIGHTEEN FIGURES)

Introduction

Since the appearance of the papers of GARNER and ALLARD (9, 10) dealing with photoperiodism, there has been a widespread interest in the relation of light to plant growth. The writer began experiments with maize and wheat in 1924, and in 1925 commenced a systematic study of the growth made by various plants when natural daylight is supplemented by artificial illumination. In earlier studies, the light was kept burning from late afternoon until 10:00 or 11:00 in the evening. After a time it became apparent that most plants do not need a rest period of darkness, and that more striking acceleration of growth takes place with longer illumination, although textbooks of plant physiology usually intimate or even definitely state that light has a retarding influence on growth. This may be true if light is very bright (5), but adding to the length of day with light of moderate intensity practically always stimulates stem elongation, and with most annuals this is accompanied by flower development. Beginning in 1926, the light was turned on each afternoon and kept burning until 8:00 the following morning. Since December, 1928, in all experiments the lights have been burned continuously, night and day. Progress reports of the study have been made at meetings of the Southwestern Division of the American Association for the Advancement of Science in Santa Fe (1927) and Flagstaff (1928), and a brief statement (15) has been published.

Earlier literature on the relation of light to plant growth has been well summarized by MACDOUGAL (11). More recent papers are noted by ADAMS (1-4), who found increased growth in height of most species when given supplementary artificial illumination, and by TINCKER (17, 18), who holds that "readiness to flower" is dependent

on the proper carbohydrate-nitrogen ratio, and that this is influenced by length of day, whether natural or artificial.

There is considerable difference in results of various workers who have experimented with lengthened daily illumination, even when methods employed were essentially the same. Probably apparent contradictions result from the use of plants with widely different inherent qualities, some responding readily to the longer light period, and others being so established in their reactions that no profound changes can be brought about. With the thought of using plants of somewhat like nature, the present study has been confined to a single family, although the writer has also undertaken work with many other genera and families.

Material and methods

The uniform procedure has been to plant seeds in 5-inch pots (at least four pots for each species), and when the seedlings were well established to place a part of the material under electric lights, and to put the rest a few feet away on the same greenhouse bench but shielded from the direct rays of the lamps. All plants received full natural illumination, while those of the experimental series had, in addition, light for 24 hours per day from two 100-Watt Mazda lamps suspended in a reflector above them. The lamps do not produce any measurable increase of temperature. Use of a foot-candle meter at night showed the light to be from 10 to 20 foot-candles on the various experimental plants, a few of the tallest receiving twice this illumination at their growing tips. The controls, being in the same room, were subject to faint diffuse light at night, but measurement showed that this was less than one-half candle. There was no indication that such very weak light had any influence on the growth of the check plants. The soil used was a sandy loam; all plants were carefully tended. A temperature of 60°-80° F. was usually maintained. The less vigorous seedlings were pulled up and from four to eight plants left in each pot, except with larger species when only two or three were permitted to grow. In recording measurements the averages have been used; for anatomical study the most advanced individuals have been taken, and these have been compared with the most advanced of the control series.

Growth, flowering, and external appearance

Flowering occurred earlier in the plants given supplementary illumination. Often the stems of the experimental plants were slender and weak, sometimes decumbent; in all cases roots showed poor development. The plants were at every stage taller than the controls. Table I summarizes observations on ten species. It is seen that artificial illumination forces quickly into bloom during the short

TABLE I
RECORD OF PLANTS GROWN WITH SUPPLEMENTARY ARTIFICIAL LIGHT

SPECIES	DATE PLANTED	EXPERIMENT STARTED	PLANTS BLOOMED	CONTROLS BLOOMED	ACCELERATION IN DAYS	REMARKS
<i>Agrostemma coeli</i>	11/8/30	11/25/30	1/10/31	4/18/31	98	Controls did not bloom until lengthening days of spring; experimental plants decumbent
<i>Dianthus barbatus</i>	12/28/30	1/18/31	3/18/31	5/2/31	45	Experimental plants weak; leaves pale
<i>D. caryophyllus</i>	9/16/28	9/30/28	2/10/29	3/3/29	21	All plants sturdy, much alike
<i>D. chinensis hedderwighii</i>	12/25/27	1/4/28	3/18/28	4/21/28	34	Experimental plants procumbent
<i>D. plumarius</i>	11/27/30	2/17/31	4/5/31	5/1/31	26	All plants much alike
<i>Gypsophila paniculata</i>	12/25/27	1/4/28	2/12/28	3/18/28	36	Experimental plants look like different species; leaves small and thin
<i>Saponaria multiflora</i>	1/18/31	2/2/31	3/12/31	4/28/31	46	All plants much alike
<i>Silene acaulis</i>	4/5/28	11/22/28	Stems and leaves of experimental plants long and slender; none of plants bloomed
<i>S. inflata</i>	3/18/29	3/31/29	7/18/29	Leaves of experimental plants small; controls did not bloom the first season
<i>Viscaria viscosa</i>	11/15/30	11/27/30	1/10/31	5/10/31	120	Leaves of experimental plants very small; controls did not bloom until lengthening days of spring

days of winter *Agrostemma coeli*, *Dianthus barbatus*, *Saponaria multiflora*, and *Viscaria viscosa*. Although not used in these experiments, the common weed *Alsine media* was observed to be unaltered by the artificial light, although reported (13) to develop well marked hairiness when grown close to a 200 candle-power lamp. Not one of the species used showed any modification in pubescence or anthocyan development, but the vegetative parts often exhibited a degree of paleness. Flowers were exactly the same as under ordinary greenhouse conditions.

Anatomy

Agrostemma coeli (figs. 3-6) was studied when 96 days old, the experimental plants being under electric light for 79 days and until

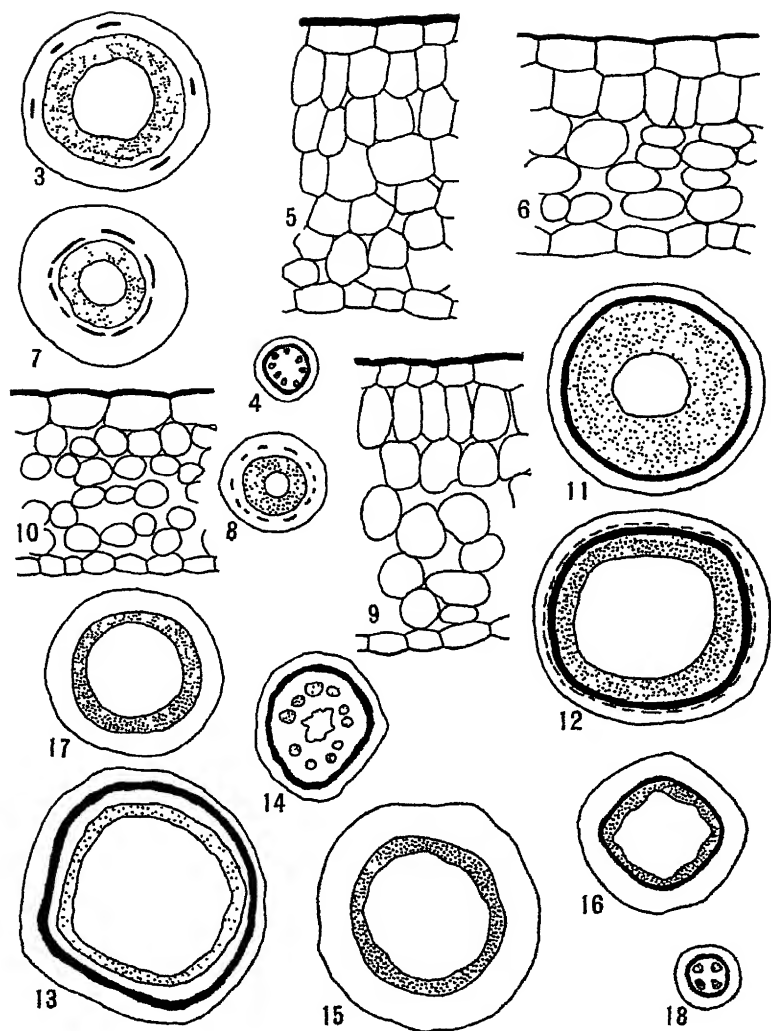
in full bloom. In cross-section of the third internode of the stem the experimental plants have a diameter 41 per cent that of the controls; the pericycle forms a continuous cylinder of lignified sclerenchyma instead of having, as the controls, scattered groups of sclerenchyma cells; vascular tissue is found in about twelve separate bundles; starch is absent from pith and cortex. Leaves of experimental plants are thin, about 66 per cent of thickness of controls.



FIGS. 1, 2.—Fig. 1, *Dianthus barbatus*, 80 days old: experimental plant at right, control at left. Fig. 2, *Viscaria viscosa*, 56 days old: experimental plant at right, control at left; $\times 1:3$.

Dianthus barbatus (figs. 1, 7-10) was studied when 80 days old, the experimental plants being under electric light for 70 days and until in full bloom. In cross-sections of the third internode of the stem these plants have a diameter 51 per cent that of the controls; both sets of plants have interrupted sclerenchyma in the pericycle with beginning of cork formation; leaves of experimental plants are thin, being 78 per cent as thick as the controls and with no true palisade.

Dianthus plumarius (figs. 11, 12) was studied when 93 days old, the experimental plants being under electric light for 42 days and until in full bloom. In cross-sections of the third internode the stems of these plants and of controls are of the same diameter, but the



FIGS. 3-18.—Figs. 3-6, *Agrostemma coeli*, 96 days old: 3, cross-section of stem of control plant; 4, corresponding internode of experimental plant; 5, vertical section of leaf of control; 6, leaf of experimental plant. Figs. 7-10, *Dianthus barbatus*, 80 days old: 7, cross-section of stem of control plant; 8, corresponding internode of experimental plant; 9, vertical section of leaf of control; 10, leaf of experimental plant. Figs. 11, 12, *Dianthus plumarius*, 93 days old: 11, cross-section of stem of control; 12, same of corresponding internode of experimental plant (circumferential row of dashes indicates beginning of cork formation). Figs. 13, 14, *Gypsophila paniculata*, 111 days old: 13, cross-section of stem of control plant; 14, same of corresponding internode of experimental plant. Figs. 15, 16, *Saponaria multiflora*, 53 days old: 15, cross-section of stem of control; 16, same of corresponding internode of experimental plant. Figs. 17, 18, *Viscaria viscosa*, 56 days old: 17, cross-section of stem of control; 18, same of corresponding internode of experimental plant. Vascular tissue shown by dotting; stereome is black. Magnification of stem cross-sections, about $\times 16$; leaf sections, about $\times 160$.

controls have twice as much vascular tissue and a somewhat thinner sclerenchyma ring of pericycle; xylem of experimental plants has larger and more numerous vessels. There is the beginning of cork formation in the lower layers of the cortex in the experimental plants.

Gypsophila paniculata (figs. 13, 14) was studied when 111 days old, the experimental plants being under electric light for 90 days and until in full bloom and fruit. In cross-sections of the third internode of the stem the experimental plants have a diameter 55 per cent that of the controls; both stems have a continuous ring of stereome, the controls possessing undissected xylem and phloem cylinders while the vascular tissue of the experimental plants is in distinct bundles and the pith is hollow.

Saponaria multiflora (figs. 15, 16) was studied when 53 days old, the experimental plants being under electric light for 38 days and until in full bloom. In cross-sections of the second internode the stems have a diameter 68 per cent that of the controls; when studied later (at 94 days) the controls had so increased in diameter that the experimental plants were only 51 per cent; a pericyclic stereome ring is present early, while this does not develop until later in the control plants. No starch was recognized in the experimental plants although abundant in the controls. Leaf structures show no differences.

Viscaria viscosa (figs. 2, 17, 18) was studied when 56 days old, the experimental plants being under electric light for 44 days and until in full bloom. In the third internode the stem diameter is only 31 per cent that of the controls; there are four small vascular bundles and a thick pericyclic stereome cylinder, whereas the controls have undissected vascular tissue and no stereome at all in the present stage. When examined a month later (at 86 days) the stems of the controls were thicker, with a strong development of xylem and the beginning of cork formation in the cortex; the experimental plants had increased only slightly in thickness and there was no change in the vascular tissue or stereome. Starch was abundant in the cortex and pith parenchyma of the controls.

Discussion

In studies to determine the influence of supplementary long-day lighting, some experimenters (5) have employed a powerful illumi-

nation nearly equal to that of sunlight; others (1, 4, 17, 18) have used such light as would be satisfactory for reading. The physiological problems involved are not exactly the same in the two kinds of experiments, yet results are often much alike. For forcing plants to develop and flower on short notice, whether for laboratory use or for sale, a knowledge of behavior with moderate light intensities is especially valuable since the necessary equipment is inexpensive and easily provided. It is shown in the present study (table I) that certain species respond quickly and very satisfactorily to illumination of the lesser intensities.

It was long ago noted by BONNIER (6) that plants given electric illumination in addition to daylight showed a somewhat etiolated appearance, having long internodes and small leaves. In the present study this condition is plainly evident in *Agrostemma*, *Gypsophila*, and *Viscaria*, but not in *Dianthus plumarius*, *D. caryophyllus*, or *Saponaria multiflora*. The other Caryophyllaceae of the experimental series exhibit an elongation of stems to some degree but their leaves are normal. Electrically lighted plants of *Gypsophila* and *Viscaria* might easily be taken for species different from their controls.

It is often held that in experiments with different day lengths, the growth of roots parallels that of shoots; in the present study the long-day exposure results in plants with lengthened stems but with short and slightly branched roots. One experimenter (7) finds that long daylight exposures favor development of roots in the radish. Many workers recognize that an accumulation of carbohydrates is accompanied by tuberization or by root thickening, hence it is perhaps to be expected that in these experimental plants, which are nearly all starch-free, the underground parts will be poorly developed. A small amount of vascular tissue, especially the phloem, is a striking feature. The slight phloem development must of necessity result in poor root nutrition and rather inadequate root growth.

Stems of the plants which have been given the extra illumination are generally weak, presumably because of their small diameter, although strengthening tissue frequently is more prominent than in

the controls. The long-night exposure to weak light no doubt causes thinness of stem, as noted in experiments by others (11, 14).

Leaves of plants belonging to the experimental series are sometimes but not always thinner than leaves of control plants, yet it has been reported (8) that, in tomatoes and peppers, leaves of plants given additional light were thicker than those of controls. In the present study the leaves of experimental plants tend to approach the usual shade-leaf type of structure, with a single layer of palisade, general loose arrangement of cells, and thin spongy layer; but instead of being a dark intense green they are usually somewhat pale. These plants resemble in general those which PFEIFFER (12) grew in light from which the violet rays were eliminated.

Explanation of the peculiarities of plant growth in the present experiment involves many factors. The plants received little violet and ultra-violet radiation because grown under glass, but the controls likewise were deprived of the short waves of the spectrum which seem to retard growth. The Mazda lamps furnished a large proportion of light of the longer wave lengths, and these rays may perhaps be responsible for some of the elongation of internodes, for it has been shown (16) that red rays favor the lengthening of stems while yellow and green rays bring about greater chlorophyll formation; it will be remembered that the plants here treated were often pale in color. Apparently the modifications in plant structure are due partly to the weak night-illumination and partly to the quality of the artificial light which was furnished.

The energy of the electric light (10-20 foot-candles) would seem almost negligible in comparison with daylight of 2000-4000 foot candles, yet this amount of light was sufficient to bring the plants out of the rosette stage and to cause early flowering. During the night hours of weak illumination, the experimental plants grew in length and used up carbohydrate food without carrying on photosynthesis to any extent. They thus became starved and could not develop the stem diameter, nor, in some species, the leaf size and thickness of the controls.

Summary

1. Eight species of Caryophyllaceous plants were grown from seed to maturity in the greenhouse, the natural daylight being supple-

mented by continuous electric light from incandescent bulbs furnishing 10-20 foot-candles.

2. The experimental plants grew taller than the controls, blossomed earlier, often had more slender stems with the vascular tissue, especially the phloem, weakly developed. Roots tended to be small and short; starch was generally absent from pith and cortex of the stem. Leaves were sometimes but not always thinner than those of the controls, and sometimes showed a single layer of palisade as in rather typical shade plants.

3. Certain species of *Agrostemma*, *Dianthus*, and *Viscaria* were brought into bloom very quickly, even during short winter days, by the use of continuous supplementary light.

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[Accepted for publication April 29, 1931]

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INCREASE OF SUGAR UTILIZATION IN SPIROGYRA BY MEANS OF COMMERCIAL FERTILIZERS

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D. J. VERDA, AND W. E. BURGE

LAVOISIER (1) was the first to show that food materials are burned in the body with resulting heat production. He also found that ingestion of food increased oxidation. RUBNER (3) showed that ingestion of meat increased heat production more than did ingestion of fats or carbohydrates. The stimulating effect of meat or protein on metabolism is due to the amino acids, the end products of protein digestion as shown by LUSK (2). SPOEHR and MCGEE (4) found that the amino acid, glycine, increased sugar utilization in *Helianthus*.

The object of the present investigation was to determine the effect of fertilizers on the rate of sugar utilization in the plant. *Spirogyra porticalis* was selected for the experiment. Large quantities of the material were collected from a nearby lake and brought to the laboratory. It was then washed in 0.1 per cent dextrose solution, and after removing the excess liquid by gently squeezing with the hands, lots of 150 gm. each were weighed out. These batches were placed in flat bottomed dishes, 20 cm. in diameter, in 600 cc. of 0.1 per cent dextrose solution. Six hundred mg. of 53 different commercial fertilizers were weighed out and boiled in 15 cc. of water.¹ After cooling, these fertilizers were added to the different batches of *Spirogyra* immersed in the sugar solutions. Portions of material to which no fertilizers were added served for controls. A small amount of sugar solution was removed from each of the dishes containing the *Spirogyra*, and sugar determinations were made according to the method of BENEDICT, immediately and after 40 hours, at the end of the experiments. Before making the determinations at the end of the experiments, water was added to make up for that lost by evaporation.

The results of an average of five series of experiments are shown in table I. By comparing the percentages of sugar used, it will be

¹ We desire to express thanks to the different fertilizer companies who so generously supplied us with the samples of fertilizers used in this investigation.

TABLE I
RESULTS FROM ADDITION OF DIFFERENT COMMERCIAL FERTILIZERS
TO SPIROGYRA IN SUGAR SOLUTION

PERCENTAGE OF SUGAR USED									
0	10	20	30	40	50	60	70	80	90
Control			32						
+ Leunasalpeter.									90
+ Arcadian Supreme Nitrate of Soda.									89
+ Urea Calcium Nitrate									
+ Plant Marvel "B"								79	
+ Calurea 34 5% Nitrogen							60		
+ Multiple Superphosphate 40%							68		
+ 2-8-10 Synthetic Nitrogen							68		
+ Swift's Red Steer 20% Superphosphate							67		
+ 20% Superphosphate							67		
+ Wizard Shredded Cow Manure							65		
+ 2-8-10							63		
+ 0-5-10							63		
+ 0-12-12							62		
+ Swift's Red Steer 0-8-24							62		
+ Wizard Pulverized Mule Manure							62		
+ 0-5-15							62		
+ Bone-Meal							62		
+ Ammonium Sulphate							62		
+ Wizard Pulverized Sheep Manure							61		
+ Sheep's Head Pulverized Sheep Manure							61		
+ 4-24-4 Multiple Strength							61		
+ 0-20-20							61		
+ 3-8-6							61		
+ V. C. 2-12-2							61		
+ Sulphate of Potash							60		
+ 4-16-20							60		
+ 0-10-10							60		
+ Ammonium Sulphate							60		
+ Plant Marvel "C"							60		
+ V. C. 4-16-4							60		
+ 2-8-16							60		
+ 2-12-6							60		
+ 2-8-16							60		
+ V. C. Superphosphate 24%							59		
+ V. C. Superphosphate 16%							58		
+ 0-12-12							58		
+ 0-8-24							58		
+ Swift's Red Steer 4-16-4							58		
+ Urea Basf 46% Synthetic N							57		
+ V. C. 2-10-6							57		
+ V. C. 3-12-4							57		
+ V. C. 4-24-4							57		
+ Swift's Red Steer 2-16-2							57		
+ Armour's Big Crop 24% Superphosphate							56		
+ Sure Winner 20% Phosphate							56		
+ 29% Superphosphate							54		
+ 16-9-0							54		
+ 20% Superphosphate							54		
+ 0-0-27							53		
+ Muriate of Potash							52		
+ 2-12-6							52		
+ Wizard Pulverized Hog Manure							45		
+ 1-10-4							43		
Control			32						

seen that all the fertilizers increased sugar utilization above the controls; that fertilizers such as Leunasalpeter and Arcadian Supreme Nitrate of Soda increased it most, while Muriate of Potash, 2-12-6, Wizard Pulverized Hog Manure, and 1-10-4 increased it least.

The next experiments were carried out with the use of chemical compounds, many of which are contained in the fertilizers used. The method of procedure was the same as that already described.

TABLE II
RESULTS FROM ADDITION OF CHEMICALS TO SPIROGYRA IN SUGAR SOLUTION

PERCENTAGE OF SUGAR USED										
0	10	20	30	40	50	60	70	80	90	
Control				41						
+CO(NH ₂) ₂									85	
+(NH ₄) ₂ SO ₄						66				
+Ca ₃ (PO ₄) ₂						64				
+K ₂ HPO ₄										
+NaCl										
+KCl						53				
+(NH ₄) ₂ HPO ₄						53				
+CaSO ₄						51				
+K ₂ SO ₄						51				
+KHCO ₃						50				
+Ca(NO ₃) ₂						49				
+NaNO ₃						47				
+MgSO ₄						46				
+MgCl ₂						42				
+Na ₂ HPO ₄						42				
+CaCl ₂						41				
+Na ₂ SO ₄						39				
+CaH ₄ (PO ₄) ₂						38				
+NaHCO ₃						36				
Control						29				
Control						41				

Lots of *Spirogyra* weighing 150 gm. were placed in 600 cc. of 0.1 per cent dextrose solution in dishes similar to those previously used. To the different dishes, 600 mg. of the various chemicals listed in table II were added, after being dissolved in 10 cc. of boiling water. After adding the chemicals, small amounts of the sugar solution were removed from the dishes and sugar determinations were run immediately and after 48 hours, at the end of the experiments. The results of an average of four series of experiments are shown in table II. By comparing the percentages of sugar used by the various lots of *Spirogyra*, it will be seen that those to which such chemicals as urea, ammonium sulphate, and calcium phosphate were added used most

sugar, while those to which such chemicals as calcium chloride, sodium sulphate, and sodium bicarbonate were added, used least.

It may be noticed in tables I and II that the fertilizers and pure chemicals that stimulated sugar utilization most were those richest in nitrogen. Table III gives data taken from table II, showing a comparison of nitrogen content and stimulating effect on sugar utilization. It will be seen that urea, the richest compound in nitrogen (46 per cent), increased sugar utilization most, while calcium and sodium nitrates, the poorest compounds in nitrogen (13 and 16 per

TABLE III
RELATION BETWEEN NITROGEN CONTENT AND STIMULATING EFFECT
ON SUGAR UTILIZATION

PERCENTAGE OF SUGAR USED										
0	10	20	30	40	50	60	70	80	90	
Control...				41						
+CO(NH ₂) ₂			(46% N)						85	
+(NH ₄) ₂ SO ₄			(21% N)				66			
+(NH ₄) ₂ HPO ₄			(21% N)			51				
+NaNO ₃			(16% N)		46					
+Ca(NO ₃) ₂			(13% N)		47					

cent, respectively), increased it least; and that ammonium sulphate and phosphate, which contain intermediate amounts of nitrogen, had intermediate effects on the rate of sugar utilization.

It should be stated that the experiments reported in this paper were carried out in dim light. After certain of the experiments had been going for several hours, small portions of sugar solution were removed from the dishes and sugar determinations made. The *Spirogyra* was then removed from the dishes, and it was found that sugar utilization was greatly decreased or had practically ceased upon this removal, indicating that bacteria were not using the sugar. In what way the *Spirogyra* utilizes the sugar, whether for storage, metabolized or adsorbed, the experiments do not indicate. Apparently they do show, however, that the *Spirogyra* and not the bacteria used the sugar, and that fertilizers increased the rate of utilization.

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[Accepted for publication April 17, 1931]

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ORIGIN OF LEAF, AND ADVENTITIOUS AND
SECONDARY ROOTS OF CERATOPTERIS
THALICTROIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 423

M. DORISSE HOWE

(WITH NINE FIGURES)

According to the classification of ENGLER, *Ceratopteris thalictroides* is a member of the Parkeriaceae. This tropical amphibious form has the peculiarity of forming buds in the notches of the leaves, which rapidly develop into new plants while the leaves still form a part of the parent plant. It was these buds which furnished material for the following work. The plants were growing in the University of Chicago greenhouse. Material collected during a time of rapid growth was found most favorable for the study of origins. Formalin-acetic-alcohol proved to be a satisfactory killing and fixing agent. Sections were stained with safranin and light green.

The general anatomy of *Ceratopteris thalictroides* has been studied by Miss FORD¹. Her conclusions, however, were not of a nature to be of interest in this paper. The origin and development of the leaf were briefly described by KNY². He mentioned that the root is exogenous, but beyond stating that it is derived from a hypodermal cell he did not describe its origin.

The leaf initial is derived from the outside portion of the segment of the tetrahedral apical cell of the main axis. The first division of the segment forms a radial anticlinal wall by which the segment is divided into two unequal cells (fig. 1 a). The first division of the larger of these two cells is periclinal, giving rise to a large outer and a small inner cell (fig. 1 b). The large outer cell then undergoes an anticlinal division in a plane parallel to the upper wall of the segment. The wall formed at this division curves downward at the

¹ FORD, S. O., Anatomy of *Ceratopteris thalictroides*. Ann. Botany 26:95. 1902.

² KNY, L., Die Entwicklung der Parkeriaceae. Nova Acta K. Leop. Deutsch. Akad. Naturf. 1875.

inner edge (fig. 1 *c*). The upper cell thus formed is narrow, the lower one broad. This latter undergoes a division similar to the preceding one, except that the wall formed curves upward at the inner edge (fig. 1 *d*). By the last two divisions a wedge-shaped cell is formed, which is the leaf initial. The leaf initial divides first by a radial anticlinal wall which curves to the right or left as seen in cross-section (fig. 1 *e*). If this wall comes in first on the right, the wall curves to the left at its inner edge. When seen in face view the same wall bends to the right. The next division of the leaf initial is similar to the one preceding it (fig. 1 *f*), so that as a result of these two divi-

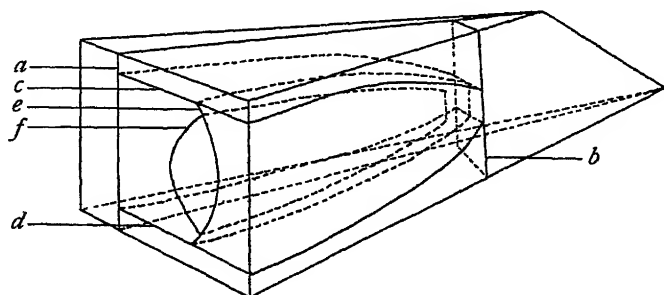
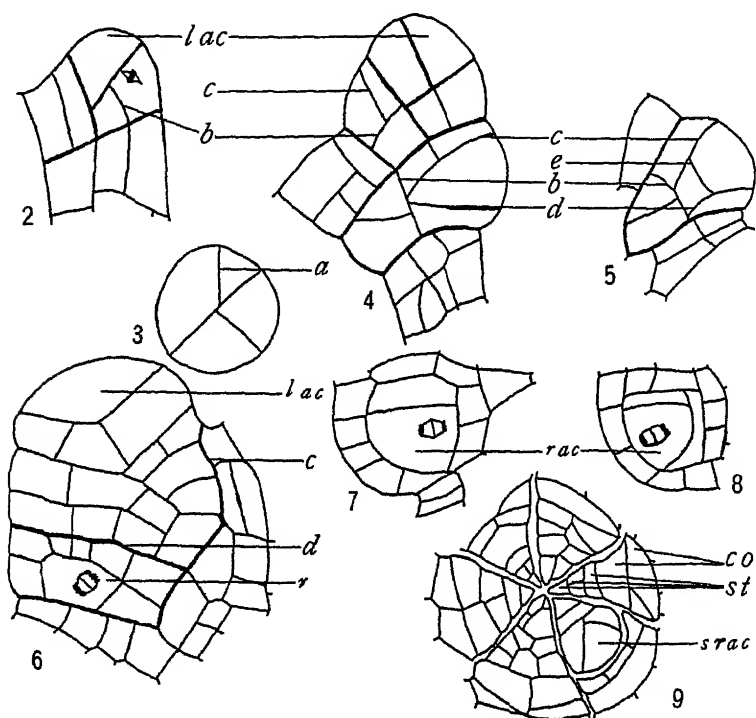


FIG. 1.—Diagram in three dimensions of segment of apical cell of main axis

sions a dolabrate cell is formed which functions as the apical cell of the leaf.

The root initial of the first adventitious root at the node is derived from the cell immediately below the leaf initial. This cell undergoes a periclinal division by which an epidermal cell is cut off on the outside. The root initial is derived from the inner cell thus formed. By one anticlinal division parallel to the upper wall of the original segment, and one periclinal division in addition to one or more radial anticlinal divisions, a mass of cells is formed from this inner cell. The root initial is the lower of the row of two cells next to the epidermis (fig. 6 *r, i*). Thus the root is not derived from the leaf itself, but from the same segment as is the leaf. The root initial may be irregular or roughly cubical. By its first three divisions, which take place in a clockwise direction on the inner faces of the cell, the root initial is transformed into a tetrahedral cell with one face toward the outside of the stem (fig. 7). The next three divisions take place

in the same planes as do the first three, and in the same order. The following division of the cell is periclinal, cutting off the root cap (fig. 8).



FIGS. 2-9.*—Fig. 2, longitudinal section of tip of main axis showing segment divisions; fig. 3, transverse section of tip of main axis; fig. 4, longitudinal section of main axis showing leaf initial; fig. 5, portion of longitudinal section of main axis showing cells derived from one segment; fig. 6, longitudinal section of leaf and portion of main axis showing root initial; fig. 7, portion of longitudinal section of main axis showing second division of root initial; fig. 8, portion of longitudinal section of main axis showing apical cell of root cutting off first cap cell; fig. 9, portion of transverse section of young root showing origin of secondary root; all $\times 275$.

* a, wall formed by first division of segment; b, second wall; c, third wall; d, fourth wall; e, fifth wall; f, sixth wall; rac, root apical cell; lac, leaf apical cell; r, root initial; rac, root apical cell, co, cortical cells; st, cells of stelar region; srac, secondary root apical cell.

The secondary root initial appears as an enlarged cell of the inner layer of the cortex of the primary root, near the apical cell. This layer finally occupies a position which is usually occupied by the

endodermis. Subsequent behavior of the secondary root initial is the same as that of the primary root initial.

The first root at a node arises from the products of the cell immediately below the leaf initial. Other roots arise soon after the first one, some of which appear to come from cells derived from the leaf initial itself.

Summary

1. In *Ceratopteris thalictroides* the leaf initial develops from the outside portion of the segment of the apical cell of the main axis.
2. The initial of the first adventitious root at the node develops from a hypodermal cell derived from the cell immediately below the leaf initial. Later roots may develop from cells derived from the leaf initial. These roots are also hypodermal in origin.
3. The secondary root develops from a layer occupying the position of the endodermis in the primary root.

Acknowledgment is due to Dr. W. J. G. LAND for his helpful criticisms in the working out of this problem.

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[Accepted for publication March, 7, 1931]

CURRENT LITERATURE

BOOK REVIEWS

Plant physiology

Botanists, and especially plant physiologists, will welcome the publication of MILLER's book on plant physiology.¹ The volume is intended for upper classmen and graduate students as well as for research workers in plant physiology. The whole field of the physiology of the green plant is covered. The order of treatment of the various subjects is logical. At the beginning are two chapters of a general nature, in which is given the description of the parts of the plant cell and the relations of the cell membranes to permeability and the development of osmotic pressure. Then follow chapters on roots; intake of water and solutes; the essential elements; transpiration; synthesis of carbohydrates; nitrogen and fat metabolism; digestion, including enzyme action; translocation of water, inorganic compounds, and organic compounds; respiration; and growth.

The book serves as a source of information, not only concerning American investigations, but of the investigations of English and continental workers. There has long been a need for a book of this kind. The European texts discuss the continental investigations but do not summarize adequately English and American research work. The material is discussed in an unbiased manner, the various theories and shades of opinion being given. Citations of the work summarized are given at the end of each chapter, the citations being referred to by years in the text. Thus the student will be enabled to read further on any subject in which he is especially interested. One is impressed with the great number of references discussed or referred to in the text. The citations at the end of each chapter cover several pages.

In the case of a book dealing with a subject which is changing as rapidly as is plant physiology, and concerning various phases of which investigators hold such divergent viewpoints, it is too much to expect that the author's treatment will be approved in all respects by plant physiologists. In regard to certain subjects, it seems to the reviewer to be inadequate or misleading. For example, it appears unnecessary to divide absorption of water by roots into absorption due to imbibition and osmotic pressure and "passive absorption," by the latter meaning that the saturation deficit set up in the leaves by transpiration is transmitted back through leaf, stem, and root to the soil, thus causing the entrance of water. It seems better to regard imbibition and osmotic pressure as involved in

¹ MILLER, E. C., *Plant physiology with reference to the green plant*. pp. xxiv+900. *figs.* 38. McGraw-Hill Book Co., New York. 1931.

the entrance of water into the root and its passage through the cortical cells of the root, both when transpiration is active and when conditions are such as to prevent transpiration or to retard it considerably. In discussing the functions of transpiration, the fact that increasing transpiration does not cause greater absorption of solutes by the root is given as evidence against the importance of transpiration in the transference of salts from the root to the leaves; but since solutes and water are absorbed by the root independently, and moved independently through the cortical cells to the root tracheae, and since a slow rate of transpiration is probably about as effective as a high one in keeping the concentration of salts in the tracheal sap low, this argument would seem to be invalid. The transpiration stream cannot carry away any more solute material than reaches it by diffusion through the root cortex. One of the most interesting phases of fat and protein metabolism is seen in their energy relations. This is rather inadequately treated in the book.

The volume is well provided with features enabling the reader readily to find information on any subject in which he is interested. There is both a subject and an author index. In addition, the table of contents is exceptionally full, each subject being divided in a detailed manner. The book is one which no plant physiologist can afford to be without.—S. V. EATON.

Principles of plant biochemistry

The first volume of a two-volume work on the principles of plant biochemistry has been published by Mrs. ONSLOW,² well known among plant physiologists and biochemists for her work on the anthocyanin pigments of plants, her practical plant biochemistry, and for her research on the oxidase system of plants. The new work gives an introduction to the carbohydrates, proteins, and the oxidizing systems of plants. The two chapters on carbohydrates are on the sugars and the cell wall. There is a chapter on oxidizing and reducing systems of plants, and then two chapters on the nitrogenous constituents. The first is entitled the plant proteins, and the second, nitrogen metabolism. The final chapter is on respiration.

The chapter on sugar considers those types found in the higher green plants mono-, di-, tri-, and tetra-saccharides, and the interrelations of these sugars in metabolism. There are useful diagrams to assist the student in grasping these relationships. The latter part of the chapter discusses the first sugar of photosynthesis (problem not solved), the glucose-fructose ratio in the plant, the concentration of hexoses, and the formation of starch and sucrose.

The polysaccharides and their congeners are considered in the chapter on the cell wall. This includes: celluloses, such as true cellulose, hydro- and oxy-cellulose, hemicellulose, pectic substances, gums and mucilages, cutin, cuto-cellulose, suberin, and lignins. One wishes that more were known of the actual

² ONSLOW, MURIEL WHELDAL, *Principles of plant biochemistry*. 8vo. pp. 336. University of Cambridge Press. 1931.

"ontogeny" of these substances; that is, the actual metabolic reactions by which they arise.

The chapter on oxidizing and reducing systems is devoted mainly to the oxygenase-peroxidase systems, tyrosinase, laccase, oxido-reductases, etc., and their relation to respiration. The reviewer thinks it would have been a better arrangement of chapters to have placed this just before the final chapter on respiration, instead of between the work on carbohydrates and nitrogenous compounds.

The chapter on plant proteins includes the albumins, globulins, prolamins, and glutelins, with tables listing those that have been isolated. There is also a table of hydrolytic products of the proteins. Under nitrogen metabolism the author presents a general summary, and specific accounts of metabolism in seedlings, ripening seeds, in starved leaves and shoots, etc. Protein synthesis is discussed, also the decomposition of amino acids by oxidation, synthesis of amino acids, and a survey of recent studies of nitrogen metabolism. The chapter closes with a consideration of purine metabolism, proteolysis, and the amino acids thus produced.

The final chapter deals with the metabolism and fermentation by yeast, and the respiration of higher plants. Here the author considers the respiratory substrate, the relation of phosphate to respiration, the dismutation of methylglyoxal, acetaldehyde as an intermediate product, and the coenzymes of the various steps. The fate of acetaldehyde, the part played by molecular oxygen in the process, and oxidative anabolism are discussed in the final sections.

While the work seems choppy in places, and not thoroughly digested, it is a valuable summary, particularly of the English and continental European work. Eleven bibliographies are provided for wide reading in that field. The second volume will be awaited with interest.—C. A. SHULL.

Handbook of plant analysis

Another excellent handbook for the research plant chemist and physiologist has just appeared. Under the leadership of KLEIN,³ the methods of examining plant materials have been brought together by a group of about a dozen collaborators. The first volume of the set is devoted to the general methods of examination for quantitative information along many lines, rather than to the methods of organic analysis for the materials of metabolism. The latter phase of analysis will no doubt be covered in the succeeding volume.

The introductory section deals with the problems of testing reagents for purity. These reagents are listed alphabetically for ease of reference, and the impurities frequently found in them are mentioned, with methods of detection and removal. Following this section, the general methods of examination of plant materials are presented in seventeen sections, the first of which takes up such

³ KLEIN, G., *Handbuch der Pflanzenanalyse*. Vol. I. 8vo. pp. xii+627. Julius Springer. Vienna. 1931.

problems as weighing, incinerating, heating, cooling, evaporating, concentrating of extracts, drying, recrystallizing, washing, stirring and shaking, clearing and decolorizing, etc. The next section gives methods of filtration, centrifuging, ultrafiltration, dialysis, electrodialysis, extraction by use of solvents, distillation, and sublimation. The succeeding sections cover the methods of qualitative elemental analysis; quantitative micro-analysis for the elements, determination of general group and radical characteristics ($-\text{OH}$, $-\text{COOH}$, $=\text{CO}$, $-\text{NH}_2$, etc.); molecular weight determinations and deduction of chemical formulae; gravimetric and volumetric methods; general physical methods (specific gravity, melting and boiling points, solubility, viscosity, molecular weights); optical methods (such as polarization, refractometry, interferometry, spectroscopy, spectrophotometry, colorimetry, and nephelometry); fluorometry; fluorescence; ultramicroscopy, photochemical analysis; electrical conductivity; electrometric determination of hydrogen-ion concentrations; colorimetric hydrogen-ion measurements; and calorimetry.

The foregoing eighteen sections comprise the first division of the subject, on general chemical and physical methods. The last portion of the first volume falls in the second division of the handbook, which deals with the general manipulations and complete analysis of plants. Only two sections of this division are included in volume I. The first of these takes up such manipulations as sampling, preservation, stabilization and drying of materials, grinding, expression of juice, steam distillation, and preparation of plant extracts by such processes as maceration, digestion, percolation, extraction, and shaking out. Solvents are considered in these connections. The second and final section deals with preliminary examination as to dry weight and water content, ethereal oil content, ash, and extractives; and the beginnings of the more thorough analysis, as microsublimation, preparation and testing of petrolether extracts, water extracts, and the examination of steam distillates, extracts, press juice, and inorganic constituents.

The work covers a very wide range of research methods, and will be useful to a wide circle of investigators in medicine, biochemistry, organic chemistry, pharmacology, agricultural chemistry, food chemistry, and plant physiology. Anyone who must make careful examination of plant materials of any kind will want this handbook as a guide. There are 323 figures in the text, and a rather comprehensive index. It is a welcome addition to the source books on methods of research in the botanical sciences.—C. A. SHULL.

Fruit culture

A new textbook of fruit culture has been prepared by KOBEL,⁴ which stresses the physiological side of horticultural science. The first section of the work deals with the general physiology of fruit trees, such as the absorption of water from the soil, transpiration and water transport, intake and utilization of min-

⁴ KOBEL, F., *Lehrbuch des Obstbaus auf physiologischer Grundlage*. 8vo. pp. viii+274. Figs. 63. J. Springer. Berlin. 1931.

eral nutrients, photosynthesis, deposition and use of food reserves, effects of cold and heat, and vegetative growth. The author then considers the problems of fruit bud formation and their development up to winter condition theories of causes of flower bud formation, and methods of influencing this bud development by cultural practices, such as fertilizing the soil, combining stocks and scions of differing vigor, accelerating or retarding synthesis of carbohydrates, girdling and strangling of branches, and pruning. Periodicity of bearing is briefly treated.

The third section considers the opening of flowers fruit setting from fertilization, fruit setting without fertilization, and the development of the fruit from the beginning to the ripe stage. Under fruit setting from fertilization, the author describes the normal processes of pollen development, female gametophyte, fertilization, and embryo formation to the seed stage. Departures from the normal behavior are considered, with special reference to sterility, including sterility due to morphological conditions, pollen sterility, sterility of female sex cells, formation of sterile seeds, and self- and inter-sterility of the various specific fruits, apples, pears, quinces, sweet and sour cherries, plums and prunes, apricots, peaches and almonds.

Fruit development includes the problem of the "June drop," and the physiological changes of the normal fruit during growth and ripening. The influence of external factors on ripening processes is discussed, and the influence of seed number upon fruit size and quality.

The fourth section is an interesting discussion of the relations between vegetative growth, flower bud differentiation, and fruiting. There are only about 20 pages in this section, but it is a valuable summary of this important phase of fruit physiology. The final section is devoted to the problems of improvement of fruits by breeding and selection.

The presentation is relatively clear and simple, and students will find it a valuable guide to an appreciation of the physiology of the fruit-bearing section of our economic plants, particularly the tree fruits.—C. A. SHULL.

Environment and plant development

The field of physiological ecology has been developing rapidly during the past decade, and among the European investigators in the experimental phases of the subject no one has stood higher than LUNDEGÅRDH. Perhaps his most notable contributions have been in establishing the relations existing between the rate of photosynthesis and the different degrees of light intensities. His curves showing the different utilization of varying degrees of light by sun and shade plants have become so well known that they have found their way into recent textbooks, even in America. His *Klima und Boden in ihrer Wirkung auf das Pflanzenleben* has a wide circulation and has run through two editions.

The appearance of an English translation of the second edition of this book⁵

⁵ ASHBY, ERIC, Environment and plant development. Transl. from "Klima und Boden in ihrer Wirkung auf das Pflanzenleben" by Dr. HENRIK LUNDEGÅRDH. pp. ix+330. Figs. 87. Edward Arnold & Co. London. 1931.

will serve to make his results familiar to a larger audience, and will encourage further research in similar fields. The translator seems to have done his work well and shows that he is familiar with the problems of experimental ecology. The English is clear cut and as simple as the subject will permit.

The scope of the volume may be gathered from the chapter headings: light factor; temperature factor; water factor; ecological properties of the soil; physical structure and aeration of the soil; chemical properties of the soil; soil micro-organisms; the carbon dioxide factor; and principles of experimental ecology. In every case the discussion abounds in the results of experimental research, and often the results are given in the form of tables and graphs. Many of these are original; others are taken from the results of other recent investigators. Perhaps the best portions of the work are in the discussions of light, certain phases of temperature, the interpretation of xerophytism, and carbon dioxide as an ecological factor.

The bibliography is rather extensive, but would have been much improved had the titles of the articles cited been given. There is both an author and a subject index. The latter seems carefully made and serviceable, although rather brief. Altogether the book is one that no ecologist can afford to be without, and the translator has rendered good service to ecology by making it available to a wider circle of readers.—G. D. FULLER.

Alpine plants in gardens

The mountain climber who wishes to bring a portion of the alpine landscape home with him, the botanist interested in the problems of mountain vegetation, and the landscape gardener, will all find interest and instruction in this recent book on alpine plants in the garden.⁶ While written from the viewpoint of the landscape gardener, it contains much accurate information for the botanist.

The first of the four parts into which the book is divided deals with the preparation of the rock garden and contains chapters on building the garden, the preparation and watering of the soil, and the various methods of obtaining and propagating alpine plants. There follow a series of sketches of the mountains where the flowers grow, the descriptions being largely confined to the mountain systems west of the Rockies. Photographs of mountain meadows and of alpine gardens are taken from the same western region. The third and fourth parts deal with the plants themselves. A long alphabetically arranged list contains many of our most beautiful alpine plants. The descriptions contain the scientific names and accurate information regarding the size, appearance, and habitat of each plant. To the botanist this carefully annotated list constitutes the most valuable portion of the book. There follow lists of species grouped according to habit, growth forms, and cultural suitability. These lists and the entire organization of the volume tend to make the information contained within it easy of reference.—G. D. FULLER.

⁶ McCULLY, A., *American alpine plants in the garden*. pp. 251. *Pls. 17*. Macmillan Co. New York. 1931.

Bacterial plant pathogens

Among recent additions to the literature welcome to the plant pathologist and bacteriologist, the volume by ELLIOTT⁷ will prove especially useful. A summary compilation of the data pertinent to bacterial plant pathogens has been a crying need for many years. Something was needed in the English language to supplement SMITH's *Bacteria in relation to plant diseases* and his *An introduction to bacterial diseases of plants*, and BERGEY's *Manual of determinate bacteriology*. SMITH's volumes were not designed to give a complete record of bacterial plant diseases, while BERGEY's manual by intent is merely a key to bacterial plant pathogens.

The volume contains an alphabetical list of the bacterial plant pathogens and of a few possible pathogens together with a list of bacteria usually associated with these. Under the heading of each organism are given its description, its synonymy, the symptoms incited by it, its hosts, its geographical distribution, and the literature. The literature is remarkably complete and this feature alone is sufficient to commend the volume to pathologists and bacteriologists. The names adopted in this volume are according to the system of SMITH's modification of MIGULA's system. Time only can tell whether this was a wise choice.—G. K. K. LINK.

Botanical explorations

An interesting little book has just appeared, written by PALMER,⁸ a Fellow of the Royal Geographical Society, with an introduction by the late J. ARTHUR HARRIS. This volume contains a modest account of mountain climbing and botanical exploration in the Selkirk, Cariboo, and Rocky Mountains of western Canada. HOLWAY began mountain climbing while on the botanical staff of the University of Minnesota at the age of nearly fifty, and during the remaining sixteen years of his life he climbed scores of the highest peaks of western Canada. Many of these were first ascents and in reaching them he traversed much unexplored territory. He is entitled to rank among the foremost mountaineers of America, and this volume seems a fitting memorial to a daring, original, and modest explorer.—G. D. FULLER.

Sylloge Fungorum

Volume XXV of SACCARDO's *Sylloge Fungorum, Supplementum Universale*, has made its appearance.⁹ It is devoted to Myxomycetae, Myxobacteriaceae, Deuteromycetae, and Mycelia sterilia.—G. K. K. LINK.

⁷ ELLIOTT, CHARLOTTE. *Manual of bacterial plant pathogens*. pp. vii+349. Williams and Wilkins Co. Baltimore. 1930.

⁸ PALMER, H., Edward W. D. Holway: pioneer of the Canadian Alps. pp. xiii+81. University of Minnesota Press. Minneapolis. 1931.

⁹ *Sylloge Fungorum*, Vol. XXV. *Supplementum universale*, Pt. X. Edit. ALEX. TROTTER. 8vo. pp. 1993. Pergola, Italy. 1931.

THE BOTANICAL GAZETTE

December 1931

EFFECT OF ETHYLENE ON THE RIPENING OF BANANAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 424

HERBERT S. WOITE

(WITH EIGHT FIGURES)

Introduction

The gas ethylene has many effects on animals and plants similar to those produced by ether, but differing also in many respects. It has been known for over a century and its anaesthetic and toxic properties have been understood for fifty years, but only within the last decade has it come into such prominence as ether has enjoyed for much longer. In this period it has found extensive employment commercially for the hastening of fruit ripening, both real and apparent. As is often the case with rapid development of a new aid to industry, however, practical application has outrun cautious investigation, and the literature mostly contains only qualitative experiments on the effects of ethylene on fruit ripening. Nothing is known of the way in which this stimulation of the ripening process is made effective, nor of the range within which it may be attained. Since the ripening of fruit is largely a process of enzymatic transformations, it was thought that an investigation of the effect of ethylene on the activity of the enzymes in the fruit might throw light on the mechanism of the stimulative effect. Earlier work on the ripening of tomatoes with ethylene, reported to the West Virginia Academy of Science in 1928, showed striking relations between enzyme activity and the ethylene treatment.

Since bananas had been widely advertised as showing marked response to ethylene in ripening, an investigation was begun of the effect of this gas on the enzymes in this fruit. To the writer's surprise, preliminary work showed that it was not possible to obtain appreciable differences consistently between treated and untreated lots. This raised the question as to the validity of the widely accepted fact of accelerating the ripening of bananas with ethylene, and accordingly the investigation was changed to a quantitative study of the chemical changes in the banana during ripening with and without ethylene.

The use of ethylene for ripening fruits had its inception in the discovery by DENNY (7) that the effective agent in the coloration of lemons by the fumes from oil stoves was the ethylene content of those fumes. Shortly after this HARVEY (12) announced that celery could be blanched profitably by the use of ethylene, and later in the same year ROSA (29) reported on the acceleration of ripening of tomatoes by ethylene, the first case of its use for fruit ripening. HARVEY (13) also reported that green bananas could be ripened to yellow in not more than 48 hours, by introducing one part of ethylene for every 1000 parts of air in the ripening rooms, and this at 18° C. Besides the advantage of reducing the storage time to half, the new process was said to save loss in weight through the shorter holding time, and loss from decay because of the lower holding temperature. Since the first report, HARVEY (14, 15) has published a number of popular articles on the ripening effect of ethylene on bananas and other fruits which are shipped in the green condition; but no chemical analyses seem to have been made by him in support of the observations on the apparent acceleration of ripening in these fruits.

A few others have tried the effect of ethylene on bananas. CHASE and CHURCH (5) made analyses of fruits ripened with and without ethylene, and reported no effect (except color) for citrus fruits and dates, while persimmons showed definite acceleration of ripening. The two experiments on bananas were so unsatisfactory with regard to the conditions under which they were carried out that no analyses were made of them. HIBBARD (16) has recently reported on a few experiments with bananas, and a single set of analyses is given. The

unsatisfactory nature of these analyses and of the conclusions drawn from them will be pointed out later.

There exists, then, only one published set of analyses of bananas ripened with ethylene, and these are open to question. The normal ripening process, however, has been analyzed repeatedly during the last three-quarters of a century, beginning with the work of BUIGNET (4) in 1861. The more important of these references prior to 1914 are listed by GORE (10), and will be given here only when they are pertinent. The analyses of GORE were by far the most thorough which had been made up to that time, and were made, as he states, "because an accurate account of the chemical changes for use in exact bio-chemical studies was . . . lacking." Unfortunately an error in method invalidates the results of his labors so far as sucrose and reducing sugars are concerned; and, like the majority of his predecessors, he failed to give the variety of banana with which he worked.

The best series of analyses available today is that of BOURDOUIL (3) for the Cavendish banana. THOMPSON (33) has given a few analyses for this variety also, while MYERS and ROSE (21) have published a set of analyses for the Gros Michel variety. This last set is open to considerable question, as will be shown later.

Materials and methods

SOURCE OF FRUIT.—In the present investigation only the Gros Michel variety, the ordinary banana of commerce in the eastern United States, was used. Through the kind cooperation of the United Fruit Company, one bunch of green bananas was supplied each week for 8 weeks for this work, and grateful acknowledgment is made to this company, and to Mr. J. H. LEATHERS, the manager of the Chicago office of its distributing subsidiary, the Fruit Dispatch, for courtesies extended. Directions were given to the foreman of the banana rooms to select as green a bunch as possible each Monday and send it to the laboratory. Unfortunately it was not learned at first that on Monday there were available only bananas which had been held since Saturday in the cold storage rooms, of which the greenest was delivered weekly for series D to H. Later, series I, K, and L were run on bananas delivered from new shipments as they

were received in Chicago on Tuesday, and were thus somewhat greener than the previous lots. Series M was carried out in the commercial ripening rooms of the Great Atlantic and Pacific Tea Company, and I am indebted to the manager of their warehouses in Chicago, and to the foreman of the banana rooms, for making possible this trial of commercial conditions, as a check on laboratory experiments.

METHOD OF SAMPLING.—In series D to I, hands of bananas were cut from the bunch as soon as it was received, and each hand was divided in halves, one for treatment and one for control. The hands of the bunch were numbered in Roman numerals from the top of the bunch downward, and the fingers of each hand were numbered in

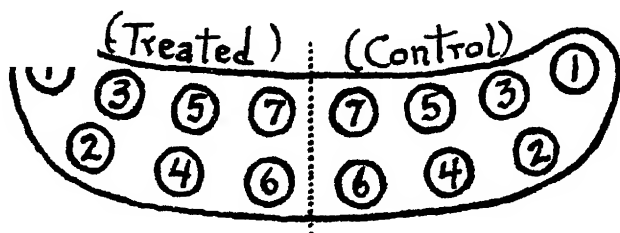


FIG. 1.—Diagram of cross-section through hand of bananas, showing scheme of numbering fingers in half hands

Arabic numerals from the outside toward the center (fig. 1). A sampling scheme was devised which seemed to eliminate variation due to the position in the hand and of the hand on the bunch in the samples for analysis. The bunches were of seven or eight hands, and hands II to VII were always used for analysis. Thus each sample consisted of six bananas, as nearly comparable as seemed possible to the bananas of the corresponding sample and to the other samples of the series. The method of sampling was to take on the first day bananas II-1, III-2, IV-3, V-4, VI-5, and VII-6, on the second day II-6, III-1, IV-2, V-3, VI-4, and VII-5, etc.

METHOD OF RIPENING.—The two sets of half hands were placed in two large galvanized iron containers of 100-liter capacity, each provided with a water seal around the cover and gas cocks at the top and bottom of opposite sides. The bananas were supported on racks of galvanized wire mesh within the cans, so that they were evenly

distributed through the volume of the containers. To one can was introduced an amount of ethylene sufficient to give the desired concentration within the can, using a mercury gas burette. This amount was 100 ml., giving a 1:1000 concentration, in series D, E, F, G, and I; and 10 ml., or 1:10,000, in series H. The other can served in all cases as a check.

In series K and L it was desired to use higher concentrations of the gas, and for these series cans of 25-liter capacity were used, as well as the larger ones. Since more than two conditions with respect to ethylene concentration were desired simultaneously in these series, it was necessary to vary the method of sampling a little. In these series, therefore, the control can received as before all of one set of half hands. The corresponding treated half hands were paired II-VII, III-VI, and IV-V, so as to neutralize so far as possible the influence of stem position on the rate of ripening (really the influence of the age of the hand); and the same finger was taken from all the half hands for each set of samples. Furthermore, the fingers of the control half hands were analyzed in corresponding pairs, instead of all together. Thus the samples for analysis are more comparable for the treated and control of any treatment on a given date than are the samples from the various treatments; but all samples on a given date are closely comparable.

The samples for series M were taken as for series D to I, but the half hands were hung in different ripening rooms, each of about 500 bunches' capacity and filled with bananas. One of these rooms had ethylene released in it each evening for 3 days, in quantity sufficient to give about 1:1000 concentration. This treatment was carried out by the engineers in charge of the ripening rooms in their regularly accustomed manner.

The temperature in series D-F and H-L was maintained constantly at $22^{\circ}\text{C.} \pm 0.5^{\circ}$, these series being run in a basement room with electric heaters thermostatically controlled. A thermograph checked the temperature regulation. Series G was run at a temperature varying between 17° and 20°C. , and series M at 18° - 19°C.

Aeration of the cans was accomplished every 12 hours during the more active period of ripening, an electric fan assisting the completeness of this operation. Analyses of the air in the cans showed that

the content of CO_2 never reached more than 10 per cent at any time, thus assuring that a plentiful supply of O_2 for normal ripening was always present. In the earlier series of experiments the CO_2 analyses were made only for the purpose of ascertaining whether the O_2 content was sufficient, but in series H to L the bananas were weighed regularly and respiratory activity determined also. Humidity within the ripening cans exceeded 95 per cent R.H., as indicated by numerous hygrograph records. Maximum-minimum thermometers laid among the bananas inside the cans showed that even during the most active period of ripening there was never a rise of temperature within the cans greater than 1°C .

METHOD OF ANALYSIS.—The samples for analysis were weighed, carefully peeled, and the weight of peel and pulp determined separately. The pulp was at once passed through a fine food chopper if it was green, or a vegetable ricer if it was ripe, and then rubbed up quickly in a mortar. Portions of 10–15 gm. were then dished out in duplicate into tared Erlenmeyer flasks of 125 ml. capacity, stoppered with tared corks, and these were at once weighed. Then 0.5 gm. of CaCO_3 was added to each flask and 50 ml. of boiling 95 per cent alcohol was poured in. After rapid mixing of the contents, the flasks were put on a hot plate and the contents boiled for 5 minutes. The whole procedure was so standardized that, except in part of one series, as explained later, the time from peeling the banana to pouring on the boiling alcohol took almost exactly 10 minutes. The flasks were cooled and stoppered, and put away in the dark until ready for the analysis.

The analysis was made for three constituents only: reducing sugars, total sugars (from which sucrose was calculated), and starch. The preserved samples were filtered off with suction, the liquid being carefully decanted from the solid matter in the flask. The residue was then treated with four successive portions of hot 80 per cent alcohol, and after the fourth decantation the solid matter was pressed onto the filter. Qualitative tests showed that the filtrate from this fourth washing was free from sugars. The alcohol on the samples was boiled gently between each two washings, to increase the certainty of the extraction of all sugars. The combined filtrates were freed from alcohol in the usual manner on the steam bath, made up

to volume, and filtered. Aliquots were taken for direct determination of reducing sugars and for acid hydrolysis to determine non-reducing sugars. For this latter operation, 1 ml. of concentrated HCl was added to 10 ml. of the original solution, and the mixture was put at 25°C. for 24-48 hours. It was then almost neutralized to litmus, made up to volume, and aliquots taken for reducing power. Non-reducing sugars were calculated by difference in the usual way and expressed as sucrose. All determinations of reducing power were made by the Bertrand modification of the Munson-Walker method. The residue after extraction of the sugars was dried to remove all alcohol and transferred to a 500 ml Kjeldahl flask. To this, 200 ml. of water and 12.5 ml. of concentrated HCl were added and the mixture heated on the steam bath under a reflux condenser for 3-5 hours, depending on the ripeness of the sample. It was then cooled, nearly neutralized to litmus, made up to volume, and filtered. Aliquots were taken for reducing power, as glucose, and the results calculated as starch. Preliminary studies, in which the action of saliva, Taka-diastase, 0.5 per cent and 2.0 per cent HCl were compared, showed that the 2 per cent HCl gave the same yields as the two enzyme procedures, whereas the 0.5 per cent HCl gave lower yields. Since the acid hydrolysis was much the simpler and more uniform method, it was adopted as accurate.

Moisture content was determined at each sampling on a sample taken simultaneously with the samples for carbohydrate analysis. These moisture samples were weighed, put in a drying oven at 105°C. for 30 minutes to stop enzyme action, and finally dried for 20 hours in a vacuum oven at 70°C.

All results have been recalculated to the basis of the original fresh weight of the banana pulp, since that mode of expression has been employed consistently by nearly all previous investigators.

Experimental results

COLOR CHANGES.—The most obvious change during the ripening of bananas is the change in color from green to yellow, and finally to brown in the overripe fruit. Table I gives briefly the salient observations on the rate of color change with and without ethylene. In no case did the control bananas lose a remnant of green persisting at the

distal end of the fruit before the close of the experiment, whereas this had long before disappeared from the treated fruits. Indeed, this persistent green tip in the controls was the most striking feature of the difference in color. The treated fruits attained a true yellow all over the peel some 12-24 hours before traces of green elsewhere than at the tips had ceased to be visible on the controls. But the difference in degree of color was small in all cases except in series L, where the controls were still green all over, and oozing latex when cut, at the end of 72 hours. In all other series it was possible for disinterested observers to distinguish treated from untreated samples at the end of 48 hours, but the difference was never very striking nor

TABLE I

RATE OF COLOR CHANGE IN BANANAS RIPENED WITH AND WITHOUT ETHYLENE

SERIES	COLOR AT START	HOURS UNTIL GREEN IS GONE FROM TIP OF FRUIT		HOURS UNTIL BODY OF FRUIT IS BRIGHT YELLOW	
		Control	Ethylene	Control	Ethylene
E	Light green	100	60	84	72
F	Light green	120	60	96	72
G	Brownish green	120	72	120	96
H	Light green	190	60	72	60
I	Dark green	168	72	120	120
K	Dark green	168	84	120	96
L	Dark green	168	84	108	120

of large degree. It was noticed that treated bananas always lost the green and developed the yellow rather uniformly over the whole body of the fruit, whereas the controls yellowed first in the middle and then increased in yellow toward the tips. Series G was run with bananas which were a brownish-green at the start and were only a dirty yellow at ripeness. It seems probable that they had been chilled in shipment, from the symptoms observed.

CARBOHYDRATE CHANGES.—The data from analyses divide naturally into three groups, according to the ethylene treatment given and the temperature used: those for 1:1000 ethylene at 22°C., those for the same concentration but at 18°C., and those for various concentrations at 22°C. All analyses were made on duplicate samples, and the average of these two is the value given in all cases. Tables II and IV give the analyses for total sugars and for starch at various

stages of ripeness with air alone and with 1 1000 of ethylene in the air at 22°C. Four separate series of analyses are involved, in three of which the bananas were no longer fully green at the start, whereas in the remaining one they were as green as it is possible to receive them in Chicago. In series D, however, both lots received ethylene

TABLE II

TOTAL SUGARS AS PERCENTAGE FRESH PULP WEIGHT AT 22°C
WITH 1 1000 CONCENTRATION OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT					
		0	24	48	72	96	120
D	{C	4.75	11.00	15.50	18.00	20.90	
	{E	5.05	10.00	14.40	18.00	20.00	
E	{C	4.20	9.62	15.25	18.55		
	{E	4.44	10.75	16.55	19.15		
F	{C	4.90	11.20	16.40	18.90	20.35	21.05
	{E	5.40	12.20	17.30	19.50	21.30	21.80
I	{C	0.92	3.72	9.50	16.80	21.75	21.50
	{E	0.90	4.15	10.00	16.55	21.15	21.10

TABLE III

TOTAL SUGARS AS PERCENTAGE FRESH PULP WEIGHT AT 18°-20°C
WITH 1 1000 CONCENTRATION OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT					
		0	24	48	72	96	120
G	{C	4.08	9.17	14.05	15.40	18.80	20.35
	{E	4.38	9.54	14.55	18.85	19.40	20.55
M	{C	1.00	3.70	6.00	10.20	14.00	17.40
	{E	0.90	4.60	8.40	12.20	16.10	19.40

through the experiment. Figs. 2 and 3 show graphically the analyses for all carbohydrates in both treated and untreated lots of series F and I. It will be observed that the various series, although showing slightly different rates of ripening, are rather uniform in showing the ethylene-treated bananas slightly more advanced in ripening (more sugars and less starch) than the controls. In series I, however, this difference is reversed. It is worthy of mention that, in every other

series than this one, the right half of the hand was always taken for the control, but in this series the left half was made the control. The small differences found consistently between treated and control halves seem, in view of the results in series D and I, to be due to in-

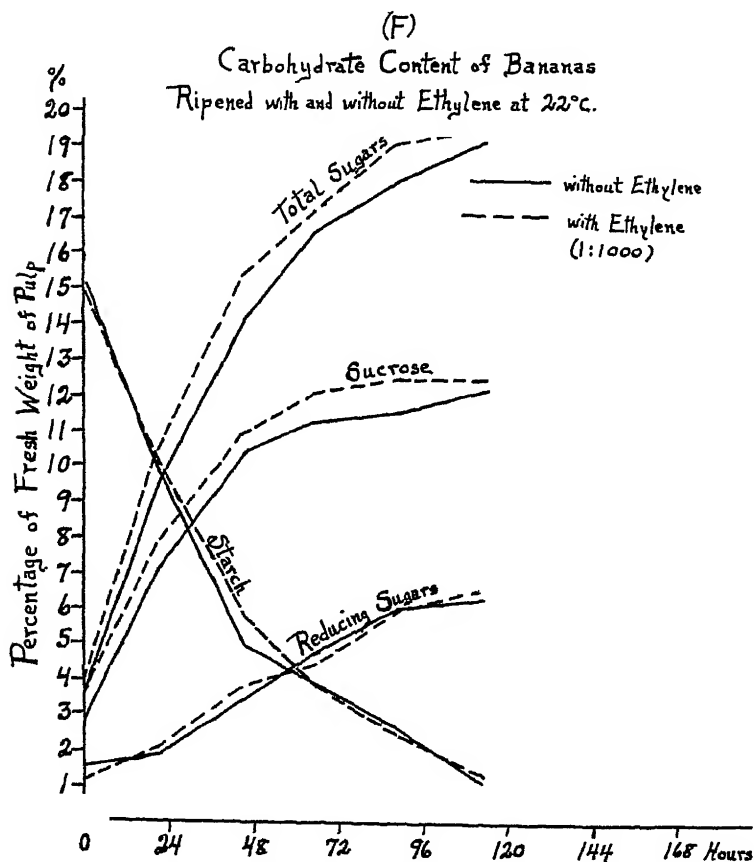


FIG. 2.—Ripening curves for bananas, series F

herent differences rather than to any treatment. In any case, the difference between control and treated fruit never even approximates that between determinations on successive days.

Tables III and V show that at 18°C. the same results are obtained as at 22°C., although the ripening process is somewhat slower at the

lower temperature. The analytical differences, therefore, are even smaller than the color differences observed, with the possibility that they are slightly but consistently in favor of ethylene. There is no difference in the ultimate sugar content of the ripe fruit.

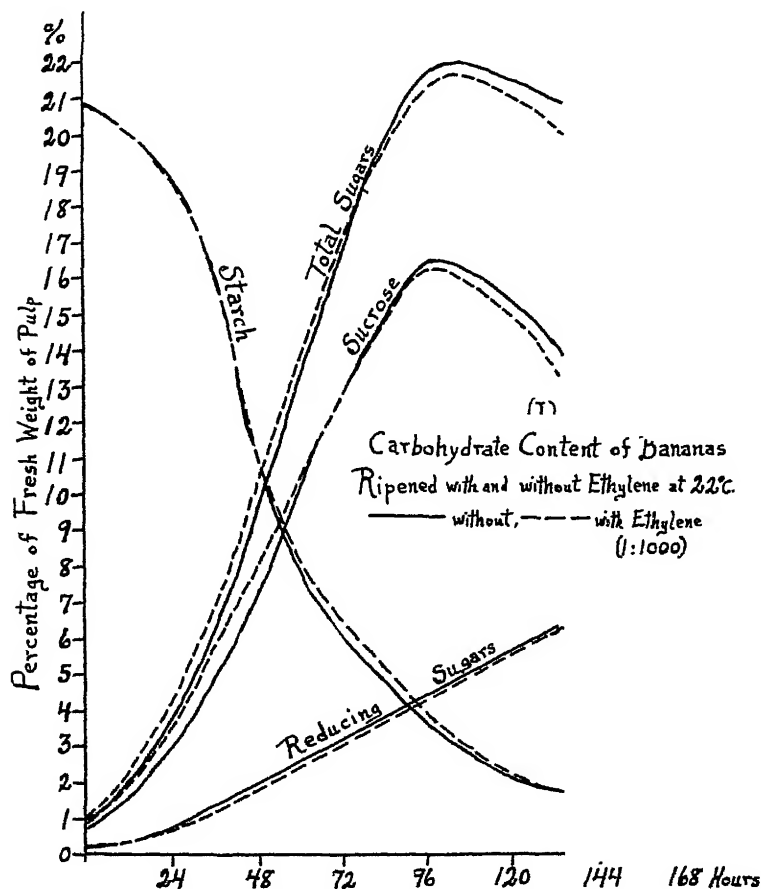


FIG. 3.—Ripening curves for bananas, series I

The results of six rather elaborate series of experiments with different concentrations of ethylene are presented in tables VI and VII. The analyses of series K show no difference between treatment with 1:250 and with 1:500 of ethylene, and the two series seem comparable with series I at 1:1000 and series H at 1:10,000 ethylene. The

difference between control and treated is the same in degree for 1:10,000 and for 1:250 parts of ethylene in the air, and the rate of ripening is the same in each case. Series L analyses show further that even 1:100 of ethylene has no different effect, either in kind or degree, from that of the other concentrations used. The differ-

TABLE IV

TOTAL STARCH AS PERCENTAGE FRESH PULP WEIGHT AT 22°C
WITH 1:1000 CONCENTRATION OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT					
		0	24	48	72	96	120
D	{E	17 80	11 00	5 90	3 30	1 40	. . .
	{E .	16 30	11 00	6 60	3 55	1 40	. . .
E	{C .	17 87	11 88	6 60	3 36	2 16	. . .
	{E . .	17 05	11 15	6 00	3 00	1 68	. . .
F	{C..	16 83	10 10	5 30	3 85	2 30	. . .
	{E .	16 17	10 35	5 95	3 63	2 20	. . .
I	{C..	20 80	19 00	11 00	6 22	3 55	2 10
	{E ..	20 75	18 60	10 80	6 62	3 95	2 20

TABLE V

TOTAL STARCH AS PERCENTAGE FRESH PULP WEIGHT AT 18°-20°C
WITH 1:1000 CONCENTRATION OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT					
		0	24	48	72	96	120
G	{C....	18 50	13 60	8 60	4 32	3 12	1 92
	{E....	18 60	13 75	8 70	4 56	3 12	1 80
M	{C....	23 05	19 80	16 40	13 00	9 20	5 50
	{E.....	23 10	19 30	15 40	12 60	8 00	4 60

ence between control and treated, however, is very great in all concentrations used in these series (fig. 4). Evidently this difference is due to some peculiar condition within the fruits of this lot, which did not obtain in any other series studied.

Although the controls of series L were greatly retarded, the ethylene-treated bananas ripened at the same rate in these series as in

TABLE VI

TOTAL SUGARS AS PERCENTAGE FRESH PULP WEIGHT AT 22°C.
WITH VARIOUS CONCENTRATIONS OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT							
		0	24	48	72	96	120	144	168
H	{Control	4 10	8 80	14 90	17 60	19 35	19 00	19 45	18 50
	{Ethylene 1:10,000	4 05	9 25	15 25	18 05	19 00	20 35	18 85	18 90
KY	{Control	1 73	8 00	14 60	17 25	19 50	20 73	20 50	20 20
	{Ethylene 1:500.	2 40	9 40	16 60	18 70	20 70	21 95	21 00	20 20
KZ	{Control	1 73	5 00	14 05	17 60	19 60	20 80	20 50	18 70
	{Ethylene 1:250.	2 40	6 80	16 27	18 60	19 90	20 60	20 30	20 00
LX	{Control	0 85	0 86	0 89	1 20	3 70	12 70	17 00	19 80
	{Ethylene 1:100...	0 95	1 85	7 00	14 50	17 50	19 00	19 90	20 50
LY	{Control	0 80	0 85	0 90	2 50	6 10	13 35	17 00	19 00
	{Ethylene 1:250.	0 85	2 50	8 20	13 90	18 40	19 50	20 40	21 20
LZ	{Control	0 85	0 86	1 40	3 20	8 60	13 80	16 50	18 70
	{Ethylene 1:500.	0 90	2 20	6 80	12 10	17 80	18 80	19 45	19 95

TABLE VII

TOTAL STARCH AS PERCENTAGE FRESH PULP WEIGHT AT 22°C.
WITH VARIOUS CONCENTRATIONS OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF EXPERIMENT							
		0	24	48	72	96	120	144	168
H	{Control.	17 40	12 55	8 05	4 20	3 15	2 15	1 55	0 90
	{Ethylene 1:10,000	17 95	12 30	7 50	3 75	2 75	1 80	1 32	0 90
KY	{Control	19 65	13 80	7 22	4 40	2 50	1 55	1 20	1 05
	{Ethylene 1:500...	19 30	13 20	6 80	4 10	2 30	1 35	0 85	0 60
KZ	{Control	19 65	14 60	8 13	4 65	2 55	1 70	1 45	1 40
	{Ethylene 1:250...	19 30	13 60	6 75	4 30	2 10	1 30	1 10	1 05
LX	{Control	24 00	23 70	23 00	21 60	17 70	10 70	6 50	3 30
	{Ethylene 1:100...	24 00	21 90	16 50	7 70	3 90	2 50	1 70	1 20
LY	{Control.	24 00	23 60	23 10	20 60	15 50	9 00	4 70	2 60
	{Ethylene 1:250...	24 00	21 00	14 00	8 40	4 20	2 65	1 90	1 15
LZ	{Control.....	24 00	23 40	22 50	19 50	13 45	8 65	5 20	2 90
	{Ethylene 1:500...	24 00	22 00	15 00	8 60	5 05	3 50	2 40	1 70

all other lots with or without ethylene. Figs. 5 and 6 show that all the curves for sugars and starch can be superimposed rather well, although the point of ripening at which analyses were begun varies

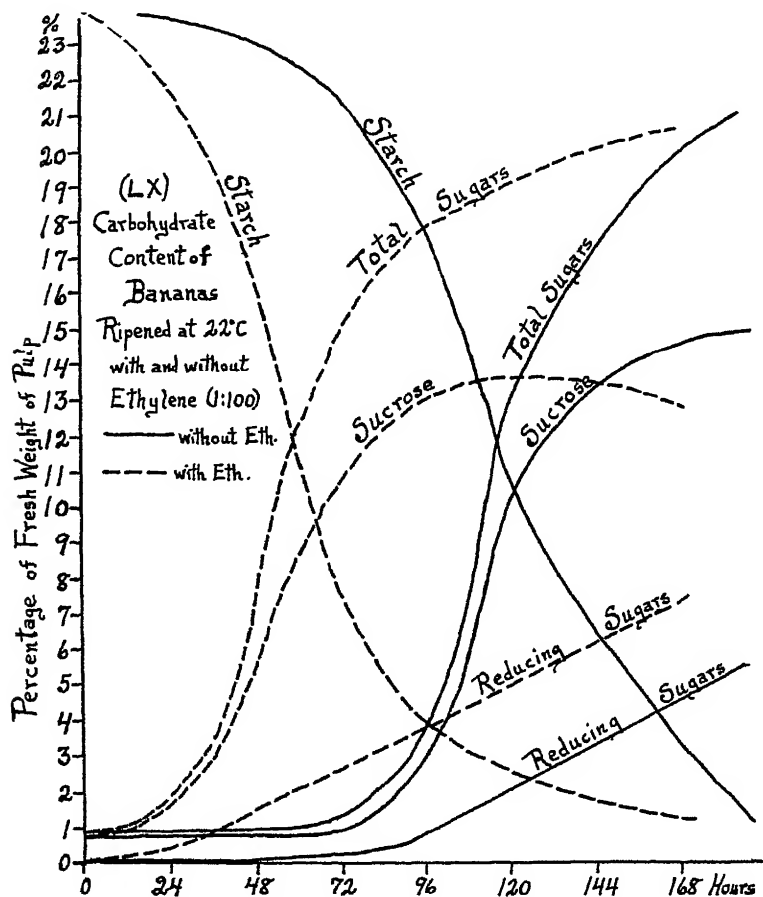


FIG. 4.—Ripening curves for bananas showing dormant stage, series LX

considerably. Indeed, this superposition makes it possible to assign a rather definite point in the ripening process as the stage of ripeness at which fruit was received, the curves for starch, reducing sugars, and total sugars being all in agreement as to the location of this point. These results emphasize the fact that in no case has ethylene

caused fruit to ripen faster than the normal, but has only caused abnormal fruit to ripen normally.

Table VIII gives the results of determinations of respiratory activity of bananas ripening in various concentrations of ethylene,

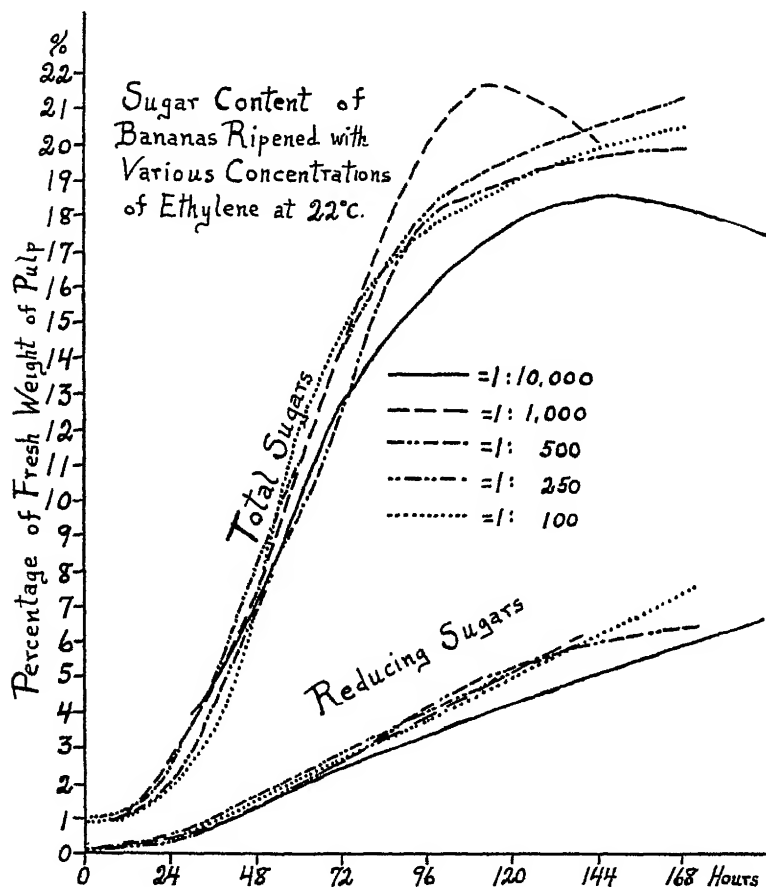


FIG 5 —Sugars in ripening bananas of several series, curves superimposed

from 1:100 to 1:10,000. The differences between rates of respiration with and without ethylene are too small in degree to be significant, except in the abnormal series L, where the same difference is found as in the ripening rate. There appears to be some suggestion that the respiratory rate increases slightly with increase in the ethylene

concentration; but fig. 7 indicates that the apparent correlation may more probably be made with the difference in rate of starch hydrolysis in the three lots of this series. It should be emphasized that in these graphs the points on the curves for starch and sugar do not

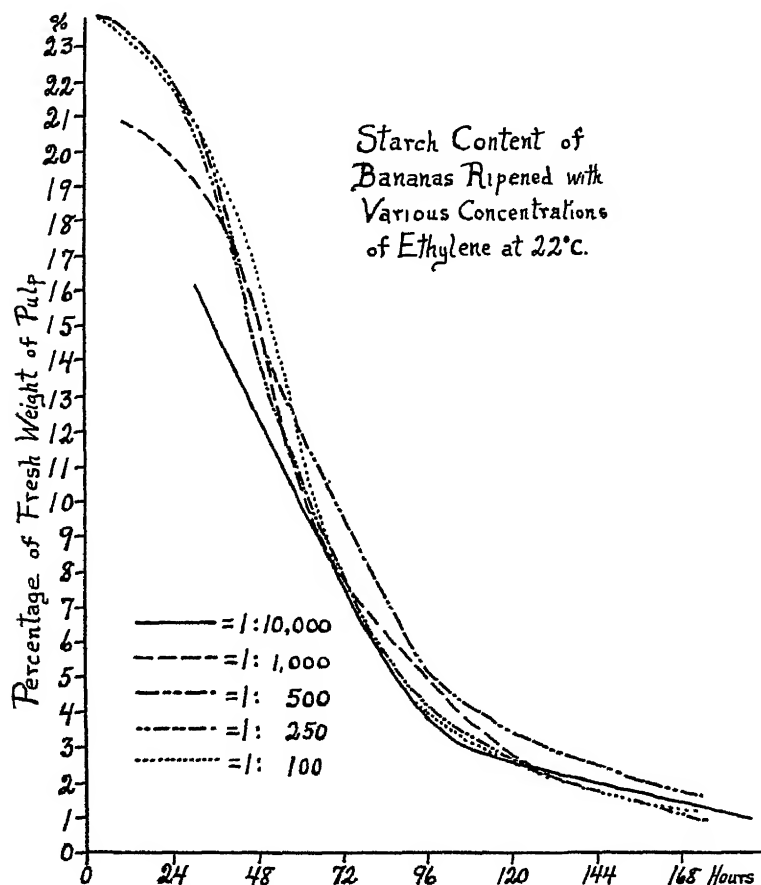


FIG. 6.—Starch in ripening bananas of several series, curves superimposed

represent the content of these substances, but the amount of change in their content during the time interval since the last previous point plotted.

Table IX contains the data on the coefficient of ripeness of the bananas at the time of analysis. The zero points of ripening have

been adjusted in this table in accordance with figs. 5 and 6, so that coefficients shown in different series are for the same stage of ripeness at the same time. The ripe bananas in series L have hardly any

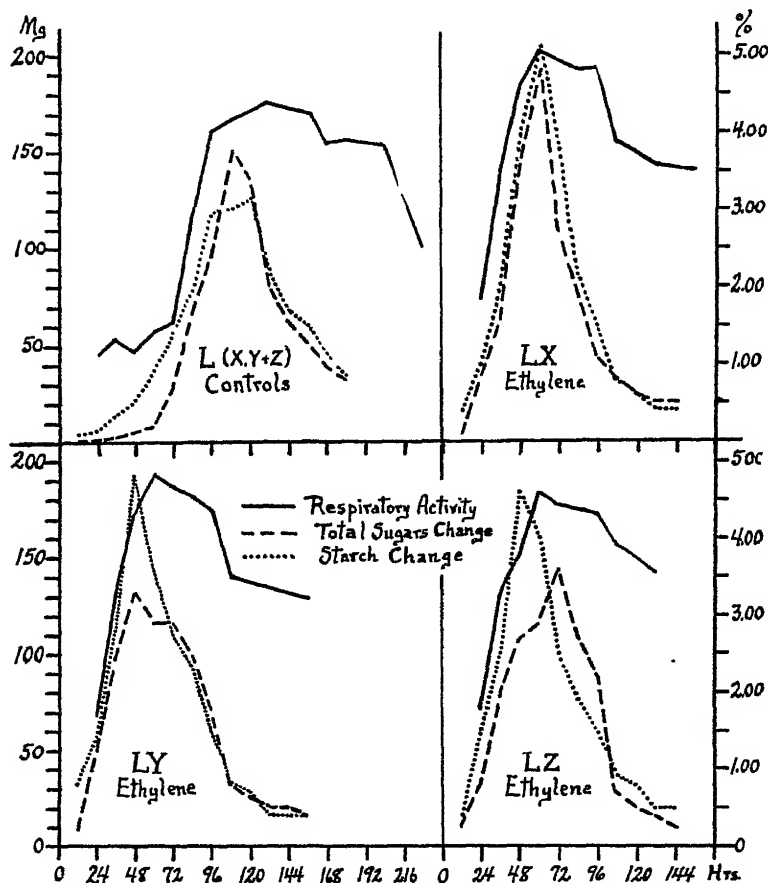


FIG. 7.—Respiratory activity versus starch and total sugar changes in ripening bananas; ordinates for respiratory activity=mg. CO₂ per kg. hr ; for carbohydrates=percentage fresh weight of pulp.

higher coefficient of ripeness than the green fruit of series K. It is evident from this table that only a negative correlation can be drawn between the value of the coefficient and the degree of ripeness of the banana. Within any given series the coefficient increases steadily in

value as ripening progresses, but the rate of increase is hardly the same in any two of the series.

TABLE VIII

RATE OF RESPIRATION AT 22°C. WITH VARIOUS CONCENTRATIONS OF ETHYLENE

SERIES	TREATMENT	MILLIGRAMS CO ₂ PER KG-HR. DURING INTERVAL IN HOURS AFTER STARTING							
		0-24	24-48	48-72	72-96	96-120	120-144	144-168	168-192
H	Control	95	132	168	120	136	.		
	Ethylene 1:10,000	91	130	170	130	132	.	.	
I	Control	75	191	187	180	127	127		
	Ethylene 1:1,000	77	195	188	179	132	128		
K	Control			180	163	112	176	98	
	Ethylene 1:500			188	140	101	126	122	.
	Ethylene 1:250			173	138	102	126	126	.
L	Control	45	50	60	138	176	171	155	153
	Ethylene 1:100	74	162	200	192	142	130	..	.
	Ethylene 1:250	68	151	190	178	135	128	.	.
	Ethylene 1:500	71	141	180	172	142			.

TABLE IX

COEFFICIENT OF RIPENESS AT 22°C. WITH VARIOUS CONCENTRATIONS OF ETHYLENE

SERIES	TREATMENT	HOURS AFTER START OF RIPENING							
		0	24	48	72	96	120	144	168
F	Control.			1 29	1 44	1 55	1 62	1 67	..
	Ethylene 1:1000			1 34	1 50	1 62	1 67	1 72	.
H	Control.		1 25	1 34	1 41	1 47	1 52	1 59	1 65
	Ethylene 1:10,000		1 30	1 38	1 44	1 50	1 56	1 62	1 67
I	Control		1 37	1 48	1 57	1 65	1 72	1 77	..
	Ethylene 1:1000		1 36	1 44	1 53	1 62	1 69	1 77
KZ	Control.		1 50	1 58	1 65	1 73	1 82	1 91	1 98
	Ethylene 1:250		1 50	1 59	1 67	1 77	1 87	1 97	2 04
LZ	Control.	1 23	1 30	1 35	1 37	1 38	1 43	1 50	1 56
	Ethylene 1:500	1 23	1 32	1 38	1 45	1 47	1 49	1 50	1 56
M	Control.	1 30	1 33	1 36	1 39	1 43	1 46
	Ethylene 1:1000	1 30	1 34	1 38	1 41	1 44	1 46

Discussion

COLOR CHANGES.—HARVEY (14) has asserted that with 1:1000 ethylene the greenest bananas can be ripened to yellow in 42-48 hours at 18°C., and has published a color plate showing the control still nearly green, with only a trace of yellow, while the treated banana is so yellow-ripe that the peel is breaking. For fruits which are not fully green when treatment starts, a still shorter time is stated to be required for the same result. Even allowing for the fact that "ripe" to the jobber means only "yellow enough to sell," it has been impossible to confirm these statements in the repeated experiments here reported. Table I shows that even with bananas no longer fully green, at least 60 hours were required; and in all other cases 72 hours was the minimum time for the development of a full yellow color with ethylene, regardless of concentration, and at 22°C. Still longer was needed at 18°C. For really green bananas, 100 hours in ethylene was needed for full yellowing.

These observations were made under laboratory conditions, but they were confirmed and enlarged in the experiment conducted at the Atlantic and Pacific warehouse, series M. At the end of 24 hours the treated and control lots both appeared to be still as green as at the start. After 60 hours the treated lot was slightly more yellow than the controls, but both still showed considerable green in the peel. Examination of the hundreds of bunches which had been ripening simultaneously in the two rooms, with and without ethylene, showed that this slight difference was true in general for the whole contents of the two rooms. Some bunches in each room were still quite green, and some were rather well yellowed, but the average of the two rooms was just about represented by the two experimental lots. So small was the degree of difference that the writer asked the experienced foreman of the banana department to express an opinion, and he agreed that the difference was of very small degree but that it was slightly apparent. There was no question at all, however, that after 60 hours of 1:1000 ethylene treatment at 18°C. (and the engineer in charge gave assurance that the ethylene had been duly administered), many bunches of bananas hardly showed any definite yellowing at all.

CARBOHYDRATE CHANGES.—With regard to the changes wrought within the banana by the treatment with ethylene, it has been stated (14) that a number of fruits, including bananas, are increased in sugar content by 20–30 per cent, and (15) that “fruits ripened with ethylene taste sweeter than those ripened with heat alone.” The present analyses would indicate that color differences of the degree found by HARVEY should correspond, not to 20–30 per cent, but to 100–200 per cent of difference in sugar content. In the analyses of many series of experiments, with various concentrations of ethylene, no difference greater than 10 per cent has been found between treated and control, except in the abnormal series L; and there it was still 300–400 per cent after 100 hours, a phenomenon apparently distinct from any that HARVEY had in mind. Untreated fruits always reached the same ultimate content of sugars as the treated ones, even when doing so more slowly. As to greater sweetness of fruits ripened with ethylene, as opposed to those equally ripe but untreated, the data for the sugars lend little support to the theory. Differences in the quality of treated and untreated fruits are not subject to exact measurement, and the writer can only say that although he sampled the ripe fruits of treated and untreated lots of every series, he could never detect any difference in flavor or quality.

The data which HIBBARD (16) has published are unfortunately those for a single experiment only, which he admits to have been the most striking of all those he performed. In this case two bunches of bananas of the same apparent greenness were compared, one in ethylene and the other in air, at 18°–20°C. As will be seen from fig. 8, both bunches were entirely green at the start, with only about 0.5 per cent of total sugars. The bunch treated with ethylene ripened at a rather normal rate, although more slowly than any in the series here reported. Even series M, at the same temperature, had reached 8 per cent of total sugars in 48 hours, while HIBBARD found only 6 per cent in his treated bunch after 64 hours. But the control bunch had made no more progress toward ripening in 10 days than was made normally in the present series in the first 24 hours. Even the controls of the series L were riper after 4 days than those of HIBBARD after 10 days. Furthermore the total sugars in his analyses never reached more than about half of the maximum value attained con-

sistently in the present investigations. The data given by GORE and by BOURDOUIL are contained in fig. 8 also, and confirm the normality of the present data. Evidently the bananas used by HIBBARD were far from normal, but it would not be safe to assert that the differ-

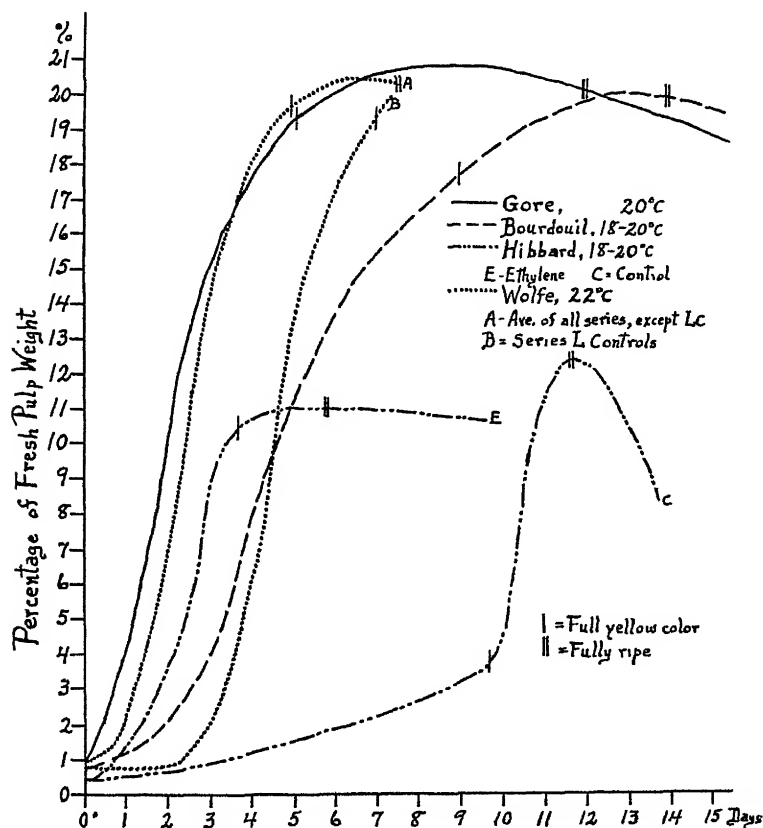


FIG. 8.—Curves of total sugars in ripening bananas as given by various investigators.

ences observed were due to the ethylene treatment. The present analyses prove conclusively that bananas from one bunch, no matter how closely similar in aspect or even in actual chemical analysis, can never accurately be compared with those of another bunch; whereas bananas from the same bunch, if properly selected, will give com-

parable results in differential treatment. Only with such comparable material can trustworthy data be obtained in studies of the effect of different treatments.

There is, however, one undoubted case of acceleration of ripening by ethylene, and that is series L of these experiments. The lots which received ethylene differed no whit in their rate of ripening from those of other series whether treated or not; but the controls showed that ethylene accelerated the ripening process greatly over what it would have been without the gas. Since the controls showed a normal ripening curve once the process had begun, it is evident that the only effect of the ethylene was to cause this process to commence at once instead of delaying several days. If it only initiates the ripening process, it is not unreasonable that bananas already started to ripen should show no acceleration of ripening due to the ethylene treatment. HARTSHORN (11) has suggested that the failure to obtain ethylene stimulation in any other series than L was owing to the ripening process having already begun in all the others. In series I and M, however, the initial stage was apparently the same in degree of ripeness as in series L, as indicated by total sugar content, and these proceeded normally with ripening. A more accurate way of stating the situation would seem to be that only series L had the fruit in a state of quasi-dormancy, from which it is possible for the ethylene to stimulate it.

The question is still moot, therefore, as to whether there is normally any stimulation of the ripening process of bananas by ethylene. Certainly there is none in the case of bananas which have already started to ripen at the time treatment is begun, even though the start has barely been made; although there is a more rapid and uniform development of yellow color. In one out of three bunches of very green bananas, as green as they can be procured in the Chicago terminal, there was found a "dormant" condition from which the ethylene effected a stimulation to ripening at once. But in only this one out of ten bunches, none of which had been held in storage more than two days and each of which was selected as the greenest of several hundred bunches in cold storage, was there any accelerating effect due to use of ethylene. What may be the nature of this "dormant" condition cannot be said, nor how the ethylene operated to

break the "dormancy." It is of interest to note that neither PRINSEN-GEERLIGS (24), working with fruit taken directly from the plant, nor BOURDOUIL, using fruit from the nearby Canary Islands, found any such delay in the start of ripening; and both show by their analyses that ripening had not yet begun in the fruit they used. Since no other sequence of analyses, using green fruit, has been published, it cannot be estimated how rarely this "dormant" condition is found. It might have been possible for the writer to have tested a hundred bunches before another such case of retarded ripening was found; and it would seem that, at least so far as the great inland distributing centers are concerned, there is very rarely received a bunch of bananas which requires ethylene in order to ripen promptly and rapidly. Once ripening has begun, its pace is not affected by ethylene. Whether or not the greater uniformity of yellow color resulting from the use of ethylene is sufficient to warrant its use commercially, is another question entirely; but it should be understood that such uniform yellow color is deceptive as a criterion of the stage of ripeness which has been attained.

SUCROSE AND STARCH IN RIPE BANANAS.—Special interest attaches to these carbohydrates, apart from the question of ethylene effect, because of the varying statements concerning them in the literature. REYNOLDS (27) recently summarized our knowledge on this subject by stating "the ripe pulp contains . . . sucrose . . . as little as 2 per cent or as much as 14 per cent." BAILEY (1) also cited the numerous earlier analyses made by workers of the United States Department of Agriculture as showing a range of 0.2-18 per cent sucrose. Such statements are misleading because they take no account of the methods used for analysis. MIERAN (20) showed by apparently conclusive experiments that the sucrase present in ripe bananas would quickly hydrolyze the sucrose present when the pulp was mashed, unless the enzyme were inactivated at once by heat. In spite of this information, however, many analyses since that time have been made without this precaution, as of course were also many of the earlier ones. The otherwise admirable analyses of GORE are useless, so far as sucrose and reducing sugar values are concerned, because he took no account of the very rapid inversion which takes place in mashed pulp. He was aware that some error existed in his method,

and states that "the values for sucrose are probably slightly high, and those for reducing sugars correspondingly low." Even allowing for the obvious inversion of intended meaning, the "slightly" is a matter of several hundred per cent. The same source of error invalidates the findings of THOMPSON and WHITTIER (33).

In table X are given the sucrose contents of ripe bananas as reported by a number of workers. It will be seen that rarely is the variety specified, and all too seldom is the method of analysis given. In all cases where satisfactory analytical procedure was followed,

TABLE X
SUCROSE AND STARCH CONTENT OF RIPE BANANAS AS
PERCENTAGE FRESH PULP WEIGHT

YEAR	INVESTIGATOR	METHOD	VARIETY	SU- CROSE	TOTAL SUGARS	SU- CROSE AS % OF TOTAL SUGAR	STARCH
1861	Buignet (4)	?	?	10	15	66	?
1876	Corenwinder (6)	?	?	15	21	70	?
1879	Marciano & Muntz (19)	?	M paradisiac	8	15	56	3 3
1882	Ricciardi (28)	?	?	4	50	24	13
1891	Mieran (20)	Boiling water	?	12	85	19	67
1896	Gerber (9)	Boiling water	?	10	45	20	55
1901	Leuscher (17)	?	Gros Michel	15	80	26	30
1908	Fransen-Geerligns (24)	Boiling alcohol	?	13	70	22	75
1911	Yoshimura (34)	Boiling water	?	8	40	14	65
1911	Reich (26)	Cold water	Gros Michel	5	40	10	35
1912	Bailey (2)	Boiling alcohol	?	5	50	10	50
1912	Thompson & Whittier (33)	Cold water	?	3	50	21	60
1914	Gore (10)	Warm alcohol	?	5	98	10	15
1914	Thompson (32)	Boiling alcohol	Cavendish	10	70	18	70
1917	Myers & Rose (21)	Boiling water	?	11	12	18	60
1922	Steel (30)	?	Fiji	5	64	24	60
1929	Bourdoul (3)	Boiling alcohol	Cavendish	13	11	10	70
1930	Wolfe	Boiling alcohol	Gros Michel	11-13	17-20	64-66	0 8-1 2

however, the sucrose content was found to be over 5.5 per cent. Expressing the sucrose more significantly as percentage of the total sugars found, to take care of the few cases in which very low total sugar values give a deceptive idea as to sucrose content, it is found that sucrose constitutes 55-70 per cent of the total sugars. The bulk of the analyses give about 20 per cent of total sugars, with 11-14 per cent of sucrose.

In the present studies, eight of the ten series of analyses showed a sucrose content of 11-13 per cent at "eating ripe," with a range of 64-66 per cent of the total sugar content. The other two analyses

showed 11-12 per cent of sucrose, but this was only about 56 per cent of the total sugars. In these two series of analyses, series K, an attempt was made to prepare samples for both series simultaneously, instead of sequentially, and so the samples stood for 20-30 minutes before inactivation, instead of for only 10 minutes as in all other series. Because the sucrose values were slightly lower by this method, a return was made in the three series of L to the original method. It can safely be stated, however, that the banana contains between 10 and 14 per cent of sucrose at good eating ripeness, which condition can be described either as when the skin is golden-yellow with tiny brown flecks, or when the total sugars have just passed their maximum. No explanation can be offered for the unusually low values for total sugars reported by a few workers, notably the 10 per cent found by BAILEY and the 11 per cent given by HIBBARD.

The starch content is also given for fully ripe bananas in table X. It will be seen that in the majority of the analyses the starch content is below 1 per cent. MYERS and ROSE (21) reported 3 per cent, but their analysis for the next day gave only 0.7 per cent, a change in rate of starch hydrolysis for this stage of ripeness not found by any other workers. Indeed, the curves for all carbohydrates in their series of analyses agree with those of other workers only up to the sixth day of ripening; thereafter they are so erratic that it is difficult to have confidence in them. The temperature used must have been very low, from the slow rate of ripening, and does not seem to have been controlled at all. The abnormally high starch content for ripe bananas reported by PRINSEN-GEERLIGS (24) must be attributed to his probably having used some small variety of plantain, which does not convert all of its starch. He found a 10 per cent lower water content and a correspondingly higher carbohydrate content than anyone else has reported for bananas. MARCANO and MUNTZ (19) also analyzed a plantain, as they state; and although YOSHIMURA (34) does not state the variety used, his anomalous result may perhaps be thus explained. In his experiment, however, the fruit had not become fully ripe after 21 days "in a warm place." Thus the starch content of ripe bananas, with the skins beginning to be brown flecked, has repeatedly been shown (in at least 15 analyses) to be less than 1 per cent. The only analyses showing a higher content are

subject to serious question, either as to their having been made on bananas or as to the accuracy of the determination.

In passing, it may be noted that the polarimetric studies of PRINSEN-GEERLIGS, THOMPSON and WHITTIER, and YOSHIMURA are all agreed that there are no sugars found in ripe bananas except sucrose, fructose, and glucose. One of the most tantalizing biochemical problems is how the starch of the banana is converted into sucrose in a few days, in the absence of any demonstrable amylase.

RESPIRATORY RATIOS.—The respiration data are in general agreement with those given by GORE (10), OLNEY (23), and PRINSEN-GEERLIGS (24). The rate is maximum when starch is being most rapidly transformed into sugars, and falls again more slowly as ripening progresses. GERBER (9) gave two sets of analyses, one of which makes a normal curve, while the other hardly rises and falls. The only previous work on the effect of ethylene on the respiration of bananas was done for very short time intervals only, and not through the whole course of ripening. REGEIMBAL, VACHA, and HARVEY (25) showed that when ethylene is added to the air in which a ripening banana is contained, there is quickly a great increase in respiratory activity, followed in a few minutes by a decrease below normal, and then eventually the normal rate is regained. The uniformity with which the data for the respiratory activity of bananas treated with ethylene parallel those for the controls in the present investigations indicates that after the response of the first hour was past, there was no further effect of the ethylene on the rate of respiration. And the effect during the brief stimulatory period is too small to be detected in the output of CO_2 for 12 hours. These observations seem to lend support to the theory of NORD and FRANCKE (22), that the effect of ethylene on ripening is due in part at least to the increase in permeability of the cells, but the change is only temporary.

These observations, previous and present, are all in considerable agreement for the banana, and are all different from those for citrus fruits. DENNY (8) studied the effect of ethylene on the respiration of lemons, and found that, on the day after the addition of ethylene to the air of the ripening chamber, there was a greatly increased respiratory activity. There was not, however, any effect observable during the 3-5 hours immediately following the introduction of ethyl-

ene, and there was a persistent effect for several days after discontinuing the treatment. This is in contrast with the immediate but brief response given by bananas. MACK (18) has reported finding that ethylene doubled the rate of respiration in celery, but he does not say whether this is an immediate and brief response, such as the Minnesota workers found for bananas, or a retarded but long continued response, such as DENNY found for lemons.

COEFFICIENT OF RIPENESS.—The "coefficient of ripeness" was introduced by TALLARICO (31) some years ago, as an expression of the quotient of pulp weight divided by peel weight. He noted that this quotient steadily increased during ripening, and believed that it afforded a reliable index of the degree of ripeness of the fruit. In the case of the (unnamed) variety with which he worked, small bananas with thin skins, this value ranged from 0.85 to 2.80 during ripening, a range permitting a moderate degree of sensitivity for indicating degrees of ripeness. TALLARICO made no chemical studies in support of his assumption, however, but merely employed the coefficient values to indicate the stage of ripeness of bananas whose enzyme activity he was endeavoring to determine. The term has remained in the literature, and has been employed occasionally, as by BAILEY (2) and BOURDOUIL (3), without any critical examination of the validity of the concept having been made. MYERS and ROSE (21) have recommended a related concept, the peel weight as percentage of the whole fruit, and PRINSEN-GEERLIGS (24) used the complementary concept, the pulp weight as percentage of the whole fruit, from either of which the coefficient of ripeness is immediately derived and both of which are subject to the same errors as is the first term. BOURDOUIL has pointed out that, with fruits of larger size and thicker peel than those used by TALLARICO, the small range of values for the coefficient makes it a not very accurate nor sensitive criterion of the stage of ripeness; but the present investigation seems to be the first to demonstrate the fallacy of the concept. Probably this is owing largely to the fact that almost all of the previous series of analyses have been single and unrepeatable, whereas the ten series in duplicate carried through from green to ripe in the present studies have afforded an opportunity for critical comparison. Besides the data in table IX, the writer has hundreds of determinations of the coefficient for

individual fruits, and these all demonstrate that, even for the fruits of the same hand, the value of the coefficient is not an absolute indication of the stage of ripeness; while for different bunches it possesses no absolute meaning at all. The value is so easily and quickly determined that it would be a most convenient reference point in analytical work if it were trustworthy, but it is not. Only chemical analyses can give any reliable measure of the degree of ripeness of bananas, and it is immaterial whether total sugars or starch content be determined for this purpose.

It is to be regretted that there is no such complete analysis for the Gros Michel variety as BOURDOUIL has given for the Cavendish. The present studies are believed to be reliable, and have been repeated so often as to leave little doubt of their accuracy, but they cover only the period up to full ripeness. The necessity of starting a new series of analyses each week precluded the possibility of carrying any of the series through to the last stages of ripeness.

Summary

1. Bananas ripening in an atmosphere containing 1:1000 parts of ethylene turn yellow at a somewhat more rapid rate than do controls, but the difference is only slight.
2. Such bananas show also a slightly greater increase in sugars and decrease in starch from day to day than do the controls, but the difference is even slighter.
3. Concentrations of ethylene ranging from 1:100 to 1:10,000 all seem equally effective in bringing about the small differences observed.
4. Ripe bananas have 17-20 per cent of total sugars, 10-14 per cent of sucrose, and less than 1 per cent of starch, in both the Gros Michel and the Cavendish varieties.
5. Respiratory activity of bananas treated with ethylene differs little or not at all from that of untreated ones, when 12-hour periods are considered, regardless of the concentration of ethylene used.
6. Rarely there is found a bunch of bananas which is in a quasi-dormant condition, and in this case ethylene stimulates an immediate commencement of ripening, while the controls are delayed several days in starting.
7. The "coefficient of ripeness" is unreliable, as well as insensitive.

8. Comparable results in investigations of the effect of various factors on the ripening of bananas cannot be obtained with bananas from different bunches, no matter how closely similar in aspect, but can be obtained only with bananas properly selected from the same bunch.

These investigations were begun at the suggestion of Professor CHARLES A. SHULL, who has been a constant source of encouragement during their progress; sincere appreciation of this support is expressed. A large part of the work was made possible by the award of the John Merle Coulter Research Fellowship in Botany, and the writer wishes to express his gratitude for this aid.

SUBTROPICAL EXPERIMENT STATION
HOMESTEAD, FLA.

[Accepted for publication August 18, 1931]

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ANATOMY OF THE TRANSITION REGION OF *PISUM SATIVUM*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 425

JOSEPH H. GOURLEY

(WITH TWENTY-TWO FIGURES)

Introduction

The general characters of the tribe Viciae of the Leguminosae have been the subject of inquiry by a number of investigators, both from a physiological and from an anatomical standpoint, and although rather well known, many details still remain to be determined. For the present study of *Pisum sativum*, the horticultural variety Champion of England was used. The transition region, investigated in detail, involves the hypocotyl and at least two internodes of the stem.

LESTIBOUDOIS (6) recorded the vascular arrangement in the central cylinder of *Faba equina*, which is similar to that in *Pisum sativum*. He also observed and figured the four cortical bundles, two of them consisting of fibers only and two being fibrovascular in character. The former are on the long axis of the elliptical stem and the latter on the short axis. The vascular bundles of the cortex diverged into the leaves.

VAN TIEGHEM (7) observed the triarch arrangement of the protoxylem bundles of the primary root in *Pisum sativum* with two layers of pericycle between the phloem fibers and the endodermis. He noted that the cotyledons were not opposite, but at an angle of 120° from each other, and that the first leaf occurred on the side of the epicotyl opposite the cotyledons, as it extends upward. Later (8) he studied the cortical bundles in the Viciae, noted the character of the divergence of leaf traces into the petioles, traced the course of bundles in the internodes, and recorded the origin of the two cortical vascular bundles in the cotyledonary region. He followed the course of these bundles through the cortex to the second node, where a branch from each entered the bract. The third node showed the

same plan, except that in addition a small bundle from the stele diverged out through the cortex on each side and became a part of the persistent strands. These somewhat enlarged bundles ended in the true stipules at the fourth node, and new bundles, diverging from the stele, constituted the cortical bundles of the next internode above. There was sometimes a variation from this plan: the prevailing arrangement might occur lower down than the fourth internode or not until the fifth or sixth one.

GÉRARD (3) traced the vascular system of the root and stem of several species, including *Vicia sativa*, which is similar to *Pisum*. He referred particularly to the transition region of these plants, which extended to the fourth internode in *V. sativa*, as also in *Pisum*. He noted the divergence of the leaf trace in toto from the central cylinder into the petiole, and with it the fibrous bundles which occur on the same radius, but located within the cortex. The adjacent lateral bundles of the ellipse were divided in two along a radial plane at a slightly higher level, and were laid down in such a position above the node as to form a leaf trace. Thus the arrangement which repeated the situation at the second node above was reconstructed. HÉRAIL (5) reported the typical arrangement of bundles in the first three internodes of young plants of *Pisum*, although no description was made of the root structure or of the cotyledonary node. He confirmed the work of GÉRARD, but interpreted the fiber strands of the stele as pericyclic rather than phloic.

TOURNEUX (9) pointed out many analogies in the habit of *Vicia*, *Lathyrus*, *Ervum*, and *Pisum*. Much the same anatomical structures were recorded as described by the previous workers. Comment was made upon the protostelic nature of the first internode above the cotyledonary region.

COMPTON (2) observed that seedlings of large species of the Leguminosae exhibit the stable tetrarch arrangement, while a reduction in their size has given rise to an unstable type of tetrarchy. In the Viciaeae there is a constant triarch arrangement, two xylem strands extending into the cotyledons and the third directly into the plumule. A detailed report was made of the arrangement of bundles at successively higher planes of the first node. The transition region of the first and second internodes was also described, but specific

mention was not made of the relation of exarch and endarch bundles in this region, although the course of the vascular and fibrous bundles was traced.

Methods and procedure

Plants of all ages were removed from the soil in which they were grown, and after being washed free of adhering material, the roots were immersed in a dish or beaker of basic fuchsin stain. The dye was first dissolved in a small amount of alcohol and then diluted with a considerable quantity of water. The vascular system and fibers were stained red by this procedure. The treated plants, or parts of plants, were then boiled for a short time in weak potash (KOH) solution, which softened or dissolved the parenchyma tissue and allowed the fibers and vascular strands to be teased out. The material was placed on a glass plate and kept wet with water or with an aqueous solution of glycerin to prevent rapid drying.

Fresh untreated material, especially cross-sections, were examined by the aid of the binocular dissecting microscope. Certain camera lucida drawings were made from this fresh material that served nearly as useful a purpose as those made from stained and prepared slides.

Material for slides made after the paraffin method was killed and fixed in the following solution: 50 per cent alcohol 100 cc., formalin 7 cc., and commercial acetic acid 2 cc. Safranin and gentian violet were the most useful stains.

Cotyledonary node

The hypocotyl of *Pisum* is short, and no striking difference in arrangement of the vascular bundles of the root is evident until within a few millimeters of the cotyledonary node. Then the vascular group appears suddenly widened, with connecting bands of metaxylem and a wide triangular pith. This is the beginning of the transition from root to stem arrangement, although it continues through the first and second internodes of the stem, and the characteristic stem arrangement is not present before the third internode is reached. CHAUVEAUD (1), VAN TIEGHEM (8), and in fact all writers on this subject have noted this anomalous situation in certain of the

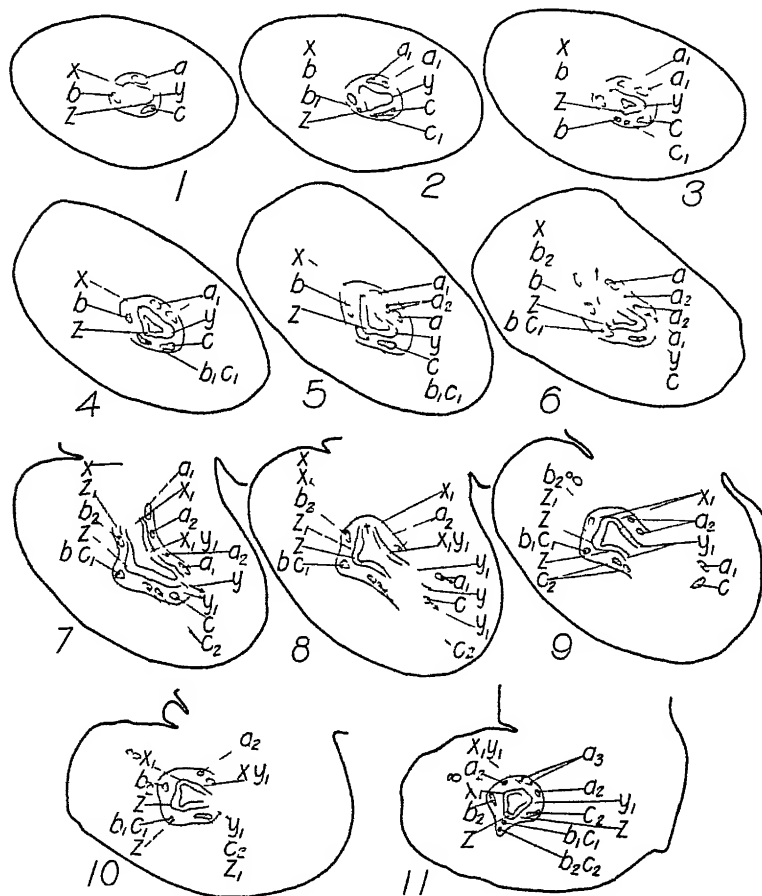
Viciae, but it is comparatively rare among other families. Histological details are lacking in most accounts.

1. FIBER BUNDLES

Since the several structures present in a representative cross-section of the root follow somewhat different courses throughout the transition from root to stem, each will be treated separately, beginning with the three bundles of fibers which cap the phloem of the primary root and hypocotyl. The divergence of these fibers can be followed in figs 1-14, which are sections drawn at successive intervals toward the top of the stem. The approximate location of the endodermis is indicated as the boundary line of the stele. Of the fiber bundles (*a b c*), portions or divergences of each extend into the cotyledons, while diverged portions which are from *b* and *c* are united over the first leaf trace (fig 5 *z*) and extend into the first internode. Divergences from *a*, *b*, and *c* form the other five fiber bundles present in the cotyledonary node, as follows. Bundle *a* (fig. 1), which is in a plane taken below the node, continues upward and occupies practically the same position in fig. 2, although noticeably attenuated; but at a somewhat higher level it is divided into two portions (fig. 3 *a₁a₁*), which become separated and occupy positions lateral to the xylem mass. Each strand is again divided (fig. 6), small strands (*a₂a₂*) being laid down as extensions from the larger strands, and continuing more or less parallel to them for a short distance, so that in transverse section all four bundles appear in a line and about equidistant from the phloem. Eventually the two strands *a₁* and *a₁* enter the cotyledons (figs. 6, 7), but *a₂* and *a₂* are left as a part of the stelar region. The strands *a₂a₂* continue upward for some distance, becoming more widely separated, and one additional strand diverges from each (fig. 11). These two latter strands (*a₃a₃*) appear as a single strand at a slightly higher level, being then located opposite the phloem of an endarch bundle (fig. 11 leaf trace *x₁y₁*); whereas the strands *a₂a₂* are located at some distance from it, each group closely capping a phloem group of the stele (fig. 12). All the strands continue this arrangement through the first internode of the plant.

In similar manner strand *b* may be followed. Beginning with fig. 1,

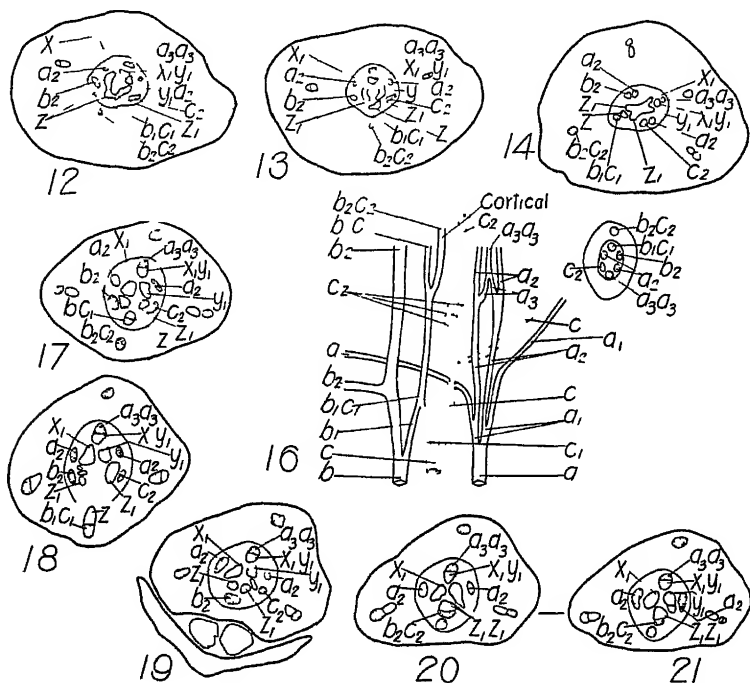
its characteristic position in the root is evident. At a slightly higher level a small portion extends from it at a sharp angle, occupying a position about one-fourth the circumference of the stele from it,



FIGS. 1-11.—Diagrammatic representation of fiber and vascular arrangement of stele and cortex of *Pisum* at successively higher levels through cotyledonary region (see text for explanation of lettering)

approximately midway between bundles *b* and *c*. At about this level, a small strand is extended laterally from strand *c*, and slightly higher still *b*₁ and *c*₁ form a single common strand *b*₁*c*₁, which continues upward for a considerable distance, when *b*₁*c*₁ is divided, the branch

(b_2c_2) occurring on the same radius and exteriorly to it, and eventually being located in the cortex (fig. 13). It is of interest in this connection that in passing through the endodermis into the cortex, this bundle at first has an independent endodermis entirely surrounding it,



FIGS. 12-14, 16-21.—Figs 12-14, 17-21, diagrammatic representation of fibers and vascular bundles cut at successively higher levels through second node of plant; leaf trace z diverges into leaf at second node, leaving gap in stele in second internode; bundles z_1 and z_2 then united as one endarch bundle at higher level, providing leaf trace z_{1-2} which supplies leaf at fourth node. Fig. 16, diagram representing perpendicular arrangement of fibers in cotyledonary region: dotted lines represent fiber group which lies on opposite side of stem; lateral divergences supply cotyledons.

while the main stelar endodermis is intact (fig. 15). At a slightly higher level the fiber bundle is surrounded by parenchymatous cells only and the endodermis cells are not differentiated. Bundle b itself is again divided, one of the two divisions passing into the cotyledon along with a vascular strand (fig. 6 x), the remaining portion b_2 continuing as one of the characteristically six fiber groups of the lower stem.

Bundle c , which is the third of the three fiber bundles characteristic of the root, occupies a position analogous to a and b in the lower portion of the hypocotyl. At a slightly higher level a small portion (c_1) on the side toward b is divided from it as already indicated. This smaller bundle (c) passes at an angle toward b_1 , and the two become a single bundle (b_1c_1) which caps a xylem group (fig. 5 z) throughout. Bundle c finally passes into a cotyledon, but previous to doing so three additional small bundles ($c_2c_2c_2$) diverge from it, all three later being merged into a single larger bundle (c_2).

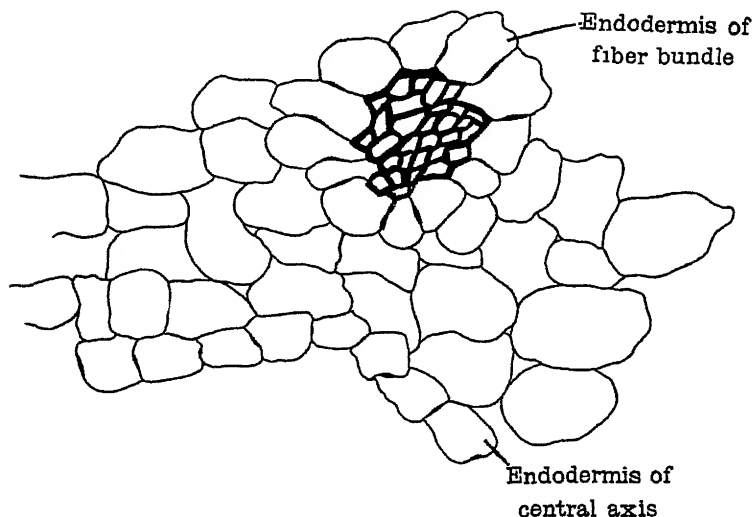


FIG 15 —Endodermal sheath about fiber bundle which is diverging from stele into cortex, below is shown section of endodermis which surrounds stele, ensheathing endodermis at slightly higher level than that shown in drawing

Thus at a low level of the first internode, near the cotyledonary node, a transverse section shows fiber bundles a_3a_3 and b_1c_1 capping the phloem of the two leaf traces (x_1y_1z), and two bundles (a_2c_2) (a_2b_2) capping the phloem of each of the two lateral bundles. Occasionally either one or both of the lateral bundles a_2 and c_2 on the one side, or a_2 and b_2 on the other, lie so closely together that they appear as one bundle or nearly so.

Referring again to bundle b_2c_2 , which is located in the cortex at a level indicated in fig. 13, its ultimate disposition may be traced.

When the leaf trace z diverges into a leaf (the details of which are described later), the fiber cap associated with it (b_1c_1) likewise diverges outward and merges with the cortical fibrous bundle (b_2c_2) at the level of leaf departure, and this new bundle subtends the vascular trace in the petiole and leaf ramifications. Above the gap in the stelar ring, which is occasioned by the departure of the leaf trace z , the adjacent fiber bundles b_1 and c_1 give off divergences which are laid down progressively nearer to one another until they finally appear as one, and occupy a position analogous to bundle b_1c_1 below the node just described. A divergence from this new bundle is laid down in an outward direction, and it may be seen in a transverse section of the stem somewhat higher up, occupying a position analogous to that of b_2c_2 in the cortex. Thus a fiber arrangement is reconstructed in the second internode, which provides for a repetition of the divergences at the fourth node.

Similarly, on the opposite side of the stem, the fiber bundle (a_1a_1) associated with leaf trace x_1y_1 gives off a divergence in the lower portion of the first internode which occupies a position in the cortex on the same radius as a_3a_3 . This bundle is not indicated at the level of the stem shown in fig. 13, but is found a few millimeters above that level (fig. 14). These two fiber bundles diverge and subtend the vascular trace of the leaf as one bundle at the third node, in exactly the same way as occurs on the opposite side of the stem at the second node. Then branches diverge from the lateral fiber bundles a_2 and a_2 , which are laid down in such a way as to provide a new fiber bundle to cap the phloem of the leaf trace of the third internode. An outward divergence from this one constitutes the cortical fiber bundle of the third internode. This procedure recurs at each node, alternating from one side to the other throughout the subsequent nodes and internodes of the plant. Fig. 16 shows in diagram the sequence of divergences through the cotyledonary region into the lower portion of the first internode.

2. VASCULAR BUNDLES

The root of *Pisum* is commonly triarch, although there are departures from this arrangement. Since not all the groups of the xylem are associated with like structures at higher levels, the three

points as seen in cross-section are designated x , y , and z respectively (fig. 1), x and y representing the bundles which finally terminate in the cotyledons, and z in the first leaf. In the root there is no pith, but in the hypocotyl, especially near the cotyledons, there is a central pith. There are a larger number of metaxylem elements in the upper hypocotyl, so that a continuous band of metaxylem borders the triangular pith and connects the groups of protoxylem. The triangular outline is much changed (fig. 5), x and y being spread outward near the two cotyledons. At the same time fewer and fewer metaxylem elements are laid down interiorly, but instead parenchyma appears (constituting pith) and metaxylem elements are laid down toward the outside, resulting in mesarch bundles in place of the exarch ones which occur just below. Finally the internal metaxylem of these two diverging groups disappears entirely, and the bundles which actually enter the cotyledons are endarch in arrangement. In this transition from the exarch through mesarch to the endarch situation there is an interspersion of parenchyma cells, so that the conventional type of one or the other is not existent at all levels.

As indicated, there is an increase in the metaxylem elements between the points x , y , and z , which are interspersed with a considerable number of parenchymatous cells, so that the sheath or envelope of the pith does not consist entirely of vessels. Slightly higher up (fig. 5) scattering protoxylem elements appear to the inside of this metaxylem band, which are finally grouped at a point opposite the first leaf trace (z), and additional metaxylem is laid down exteriorly to them. This arrangement results in the endarch bundle which supplies the second leaf or bract (fig. 7).

At the same time that the protoxylem elements are being laid down in a group which forms a part of the second leaf trace, parenchyma appears at either side of the two leaf traces, separating the latter from the more or less continuous band of vessels which envelop the pith at, or slightly above, the level indicated in fig. 7. Protoxylem cells then occur where the metaxylem bands are cut off from the leaf traces. After the traces x and y have diverged into the cotyledons, the ends of the vascular cylinder, bounding the two gaps, and represented at x_1x_1 and y_1y_1 (fig. 9), gradually become continu-

ous by the laying down of additional metaxylem. This makes possible the grouping of x_1x_1 and y_1y_1 into two continuous bundles with protoxylem points at each end, thus constituting the exarch lateral bundles of the stele as shown at the base of the first internode (fig. 13). These changes in arrangement and configuration in the cotyledonary node can be followed at the levels indicated in figs. 1-14.

In detail, the change of the first leaf trace (z) from an exarch group in the root and lower hypocotyl to an endarch one in the first internode is as follows. Toward the upper region of the hypocotyl this vascular group arches out somewhat, parenchyma cells are interspersed with metaxylem, and a little higher the protoxylem elements appear somewhat scattered, owing to the differentiation of parenchyma cells. At a slightly higher level, metaxylem fails to differentiate interiorly to the protoxylem, but elements of it are laid down outside, producing an exarch vascular bundle. Throughout there are some parenchyma cells associated with the vessels of this trace.

At the level indicated in fig. 11 there are more or less definite, although narrow, bands of parenchyma separating the elements of this leaf trace from those of the adjoining groups. This separation is higher in the structure than the separation at points x and y . The points of the lateral bands of metaxylem are therefore designated z_1 and z_1 ; and, as already noted, protoxylem cells are laid down near the point of separation and actually become associated with these points. It is in this way that two of the four exarch bundles of the lower stem are produced.

By the coalescing of the lateral groups y_1 and z_1 on the one side and x_1 and z_1 on the other, continuous lateral strands are formed. At first a rather large pith occupies the central portion of the stem, but at a higher level of the first internode metaxylem cells are laid down until the central portion is completely occupied by them. Before the summit of this first internode is reached, however, parenchyma cells are differentiated within the central portion of the stem, and the siphonostele arrangement is established, which arrangement persists throughout the remainder of the stem. Above the seventh or eighth node the pith disintegrates on maturity and the stem is hollow, except at the nodes where there are constrictions.

Stem

1. EPICOTYLEDONARY INTERNODE

The general arrangement of structures as seen in cross-section of the stem near the base has been noted elsewhere (figs. 3, 4, 7, 8), and is diagrammed in fig. 14. The characteristic arrangement of the primary xylem is much as though two somewhat flattened elongated crescents, with their flattened edges in contact, were placed at the center of the stem, the tips of each crescent being the protoxylem and the space between them being occupied by the metaxylem. Between the divergent tips at each end of the xylem mass, and separated from them by parenchymatous cells, is a small endarch bundle. Thus there appear to be four exarch and two endarch bundles in close association, the stele being neither completely protostelic nor siphonostelic in character.

The two "polar" endarch bundles on the long axis of the elliptical stem are leaf traces. One of them (figs. 1-14 2) supplies the central lobe of the trifid bract at the second node. The other is the vascular supply for the second leaf bract. Near the second node the leaf trace diverges outward through the cortex, enters the bract in toto, and represents the principal central vascular bundle of this leaf. As this divergence takes place, a narrow pith is laid down in the long axis of the elliptical stele, extending from the diverging leaf trace to about its center. The beginning of radial parenchyma rays, which later extend through the lateral xylem groups, originate before the leaf trace has entirely traversed the cortex in its outward course to supply the bract at this second node.

2. SECOND INTERNODE

A section cut immediately above the second node shows only one "polar" bundle, together with the central mass of xylem already referred to and a central pith. Opposite this remaining polar bundle is a leaf gap, which occupies a position homologous to the one which bundle 2 occupied below the node; the parenchyma of the pith and the gap are therefore continuous.

At a slightly higher level there is a reorientation of the exarch masses of xylem which border the gap together with the accompanying phloem. The central mass of xylem is no longer continuous be-

cause of the laying down of parenchyma cells, rather than metaxylem ones, in the central region, thus dividing the two exarch groups nearest the leaf trace. Parenchymatous cells also separate these adjacent exarch groups from the rest of the xylem mass, as a result of the laying down of such cells rather than metaxylem at that level. These radial divisions are not wide, but serve to separate the central mass into three groups, namely, the two exarch vascular strands bordering the gap and the remainder of the stelar xylem.

The exarch masses adjacent to the leaf gap (fig. 14 z_1z_1) now assume a different position relative to the rest of the central mass, as indicated in sections cut progressively higher up the stem (figs. 17-21). Just as parenchymatous cells were differentiated instead of certain of the vessels in the vascular bundles (z_1x_1 and z_1y_1), so similar cells occur between the tips of these two adjacent exarch masses, so that they form small vascular strands bordering the gap. Then metaxylem is laid down exteriorly, as a part of these groups, with the protoxylem points remaining interior in their relation to the former. The two strands are laid down progressively nearer until they finally appear as a single endarch bundle (fig. 20), which is the trace that supplies the leaf at two nodes above this point (the fourth one of the plant). The general effect is that of the two groups (z_1z_1) adjacent to the gap moving around at an angle of 90° , until they occur as a single vascular bundle which has become endarch rather than exarch, because of the changed relation in which the metaxylem elements are laid down. At the same time the associated fiber bundles are also rearranged, as already described. Thus endarch bundles are the only ones that occur on the side of the stem from which the first leaf trace diverged. Two exarch bundles, however, remain on the opposite side in the second internode.

In the upper region of the second internode, which is characteristically short in all horticultural varieties of *Pisum* examined, there is on the opposite side of the stem a repetition of the disposition of bundles already described for the first internode and second node. The leaf trace (fig. 14 x_1y_1) diverges outward through the cortex near the third node. It supplies the central lobe of the trifid bract of this node, leaving a gap immediately above, as occurred at the node below. At the same time that the leaf trace diverges into

the rudimentary leaf, there is a differentiation of primary tissue leading to the formation of an axillary shoot. This occurs also at the other nodes, and higher up there are primordia for flower branches laid down at an angle of about 35° with the stem.

3. THIRD INTERNODE

After the leaf trace has diverged into the bract at the third node, there is a repetition of the reorientation of the two exarch groups which immediately border the parenchymatous gap. By the process of laying down metaxylem elements to the outside of the protoxylem, and the laying down of parenchyma toward the inside, groups are produced which are endarch in character. These groups are so laid down as gradually to approach one another, finally occurring as a single vascular strand which is the leaf trace that supplies the leaf at the fifth node. With this rearrangement there passes the anomalous situation of both exarch and endarch bundles occurring together in the stem, and throughout the remainder of the plant all the bundles are endarch. The stem now assumes a more nearly four-sided shape, becoming increasingly more so in the internodes above. In two of the four corners of the stem are the fibrovascular bundles which supply the leaves, and in the opposite ones are the prominent bundles which supply the stipules.

4. CORTICAL BUNDLES

The bracts which occur at the second and third nodes of *Pisum* consist of three lobes, the central one being interpreted as an undeveloped blade and the lateral ones as undeveloped stipules. This interpretation is borne out by the vascular arrangement. At the fourth node and at all those above, a true compound blade and two large persistent stipules occur. The leaf traces, as already described, provide the chief vascular supply for the blades, and the fibrovascular bundles of the cortex provide the chief ones for the stipules.

The cortical bundles consist of two fiber groups on the long axis of the ellipse, one opposite each leaf trace, and two fibrovascular ones located at approximately right angles to the fibrous ones. One of the fiber bundles supplies the first bract and the opposite one the second bract, as already noted. The cortical fibrovascular bundles are con-

spicuous features of the stem of *Pisum*. They occur on the sides of the stem at right angles to those from which the leaves depart, and have their origin at the first node, from the edge of the vascular bun-

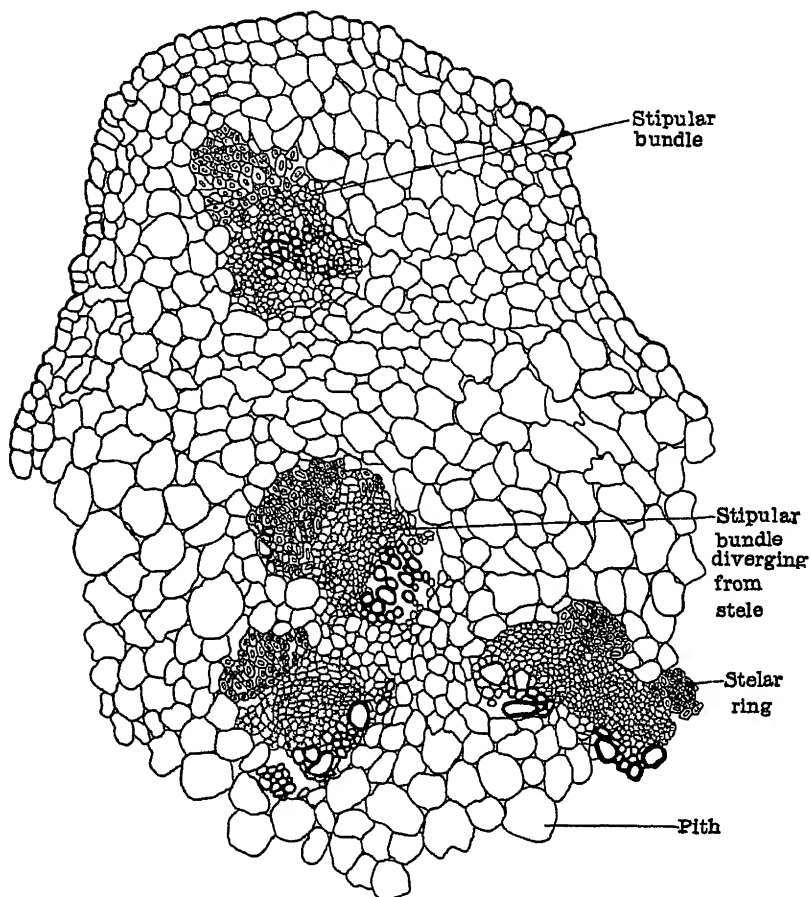


FIG. 22.—Detail of section showing divergence of bundle of stele into cortex; cortical bundle supplies stipule at sixth node.

dles supplying the cotyledons. In some cases two arise on each side, but in such event one of each pair is always small and fades out in the first or second internode. The persistent ones diverge immediately into the cortex, and at the second node a branch from each supplies the outer lobes of the bract, one from one side and one from

the other. Within the bract there is an anastomosis with the leaf trace, so that the bract appears as a single unit. Within the cortex are rather large lacunae at either side of the vascular bundles.

The main portion of the cortical bundles continues up the stem, and at the third node branches diverge to the opposite side and again occur in the outer lobes of the bracts. The main bundles of the cortex continue to the fourth node, where the first true blade and stipules occur. Here the cortical bundles diverge around the stem and enter the stipules in toto at their lowest point of divergence from the base of the petiole, and at approximately right angles to the leaf. They then proceed along the entire line of junction with the stem, ramifying branches extending throughout the stipules. The origin of the vascular bundles which supply the stipules at the fifth node is in the stele. At a short distance below the fourth node, fibrovascular groups diverge out of the stelar ring at opposite sides and at right angles to the leaf traces (fig. 22). These groups are laid down so as to form an outward course into the cortex, and extending upward through the region of the fourth node and the full length of the fourth internode. They then supply the stipules at the fifth node, where they enter them in toto, anastomose in part with the vascular elements of the leaf trace, and together with the latter form the vascular supply of the blade and stipules at this node.

Two gaps in the stele result from the divergence of the fibrovascular bundles, and are evident for a short distance above the departure of the latter. New elements are added to the central cylinder (protoxylem to the inside and metaxylem to the outside), until the gaps no longer exist at a level near the fourth node. This situation is repeated at the succeeding internodes and nodes, providing a pair of cortical bundles for each set of stipules.

Summary

1. The transition from root structure to stem structure in *Pisum sativum* is not complete until the third internode is reached. This anomaly consists in the occurrence of exarch bundles in the first and second internodes, and in a protostelic situation in at least a portion of the first internode.

2. There is a transition in the region of the cotyledonary node

from the triarch arrangement of the root to that of six characteristic vascular bundles in the first internode. These consist of four elongated lateral bundles lying on either side of the small central pith and in the direction of the long axis of the elliptical stem. They appear as two bundles since the metaxylem elements are in contact. All of the bundles are exarch, a smaller bundle lying at either end of the lateral groups, completing the series. Both of the latter are endarch. To the sides of each lateral group are two fiber groups lying about equidistant from the vascular strands, and at either end of the long axis is another fiber group, establishing the complement of six fiber groups within the stelar region.

3. The two smaller vascular groups are the leaf traces that supply the first two alternate leaf bracts. The lateral ones serve in part to supply leaf traces for the fourth and fifth nodes.

4. After the first leaf trace diverges from the stele at the second node, portions of the lateral exarch bundles are laid down progressively nearer together, until they appear as one endarch bundle above the leaf gap. The protoxylem elements occur inward and the metaxylem elements are laid down toward the outside face, producing a new endarch leaf trace. Two exarch bundles remain on the opposite side of the stem in the second internode. At the third node the same rearrangement occurs, so that in the third internode all bundles are endarch and the permanent stem structure is accomplished.

5. There are four cortical bundles in this species, one fibrous one opposite each leaf trace and a fibrovascular group opposite each lateral bundle. The fibrous ones diverge into the blades in toto, and the arrangement is established in the nodes above by divergences from the fiber groups which lie to either side of the leaf gap. The vascular ones are the stipular traces. At the second and third nodes, branches from them supply the rudimentary stipules or bracts and they end free in the first true stipules at the fourth node. Bundles diverge out of the stele in the second internode at right angles to the leaf traces to supply the next node above. This arrangement is repeated at each of the succeeding nodes.

This study was conducted under the direction of Professor E. J. KRAUS, and the writer expresses appreciation for his interest during the progress of the work.

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[Accepted for publication March 17, 1931]

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THE RELATION OF NITROGEN TO POTASSIUM IN THE NUTRITION OF FRUIT TREES

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Introduction

For a number of years it has been a common practice among fruit growers to apply nitrogen-carrying fertilizers, such as nitrate of soda and ammonium sulphate, to their orchards to the exclusion of all others. This practice has been popular because of its easily observed effects, such as increased tree growth and fruit yield, and improved color and size of foliage. Whether the effects of nitrogen alone have been desirable is a moot question. It seems to be necessary to demonstrate whether or not a fertilizer program, emphasizing the use of only one plant food element to the exclusion of others, is always the most profitable practice. It should be borne in mind that the more nitrogen applied the greater will be the drain on the mineral resources of the soil. Every pound of nitrogen used in plant nutrition requires also a certain amount of phosphorus, potassium, and other mineral elements. Continued absorption of relatively large amounts of nitrogen will cause an unbalanced nutrition when no provision is made for the renewal of the soil's supply of phosphorus and potassium, which are probably absorbed by the plant in amounts proportional to the nitrogen.

Recent investigations by the Long Ashton Horticultural Research Station in England showed that a marginal browning of the foliage of apple trees, known as "leaf scorch," is a symptom of potash starvation. It was also shown that by increasing the amount of nitrogen applied to the trees affected with leaf scorch, the condition became considerably more severe. When the proportion of nitrogen was decreased or the proportion of potassium increased, leaf scorch did not develop. Further investigations indicated that this marginal browning of leaves was caused chiefly by too little available potassium in the soil in comparison to the amount of nitrogen.

It was the purpose of this investigation to determine, in a preliminary way, the effect of altering the ratio of nitrogen to potassium

in the nutrient media of apple trees, in an effort to throw some light on the relation of nitrogen to potassium in their nutrition.

Experimentation

In each experiment quartz sand cultures were used. The method of sand cultures was employed because of the impossibility of governing the exact concentration, reaction, and composition of soil solutions, and because such a medium obviously affords a more natural habitat for plant roots than do solution cultures. The quartz sand was of a high quality and was free from organic matter and other impurities.

The following nutrient solution, which has been used successfully by WALLACE in connection with sand cultures of apple trees, was employed in this investigation:

	GM.
NaNO ₃	5 0
KNO ₃	2 0
K ₂ HPO ₄	1 0
CaSO ₄	1 0
MgSO ₄	1 0
NaCl	1 0
FeCl ₃	0 4
Tap water to make up 10 liters	

When potassium was to be omitted from, or reduced in, a nutrient solution, the salt or salts containing potassium were replaced by an equivalent amount of the corresponding sodium salt; for example, KNO₃ was replaced by an equivalent amount of NaNO₃ and so forth. Similarly, when it was desired to omit or reduce the amount of nitrogen in solution, the corresponding sulphates were used in place of the nitrates.

In this manner seven different nutrient solutions were prepared, each containing a different N/K ratio. The composition of these solutions in regard to their nitrogen and potassium content was as follows:

- Series A (110 ppm N) full nutrient
(126 ppm K)
- Series B (0 ppm N) minus nitrogen
(134 ppm K)

- Series C (115 ppm N) minus potassium
(0 ppm K)
Series D (36 ppm N) $\frac{1}{3}$ (N of full nutrient)
(126 ppm K)
Series E (323 ppm N) 3 (N of full nutrient)
(126 ppm K)
Series F (115 ppm N) $\frac{1}{3}$ (K of full nutrient)
(45 ppm K)
Series G (110 ppm N) 3 (K of full nutrient)
(368 ppm K)
Series H (tap water) check

During the first month of growth, each culture received 500 cc. of nutrient solution every 7 days. During the later period of growth, when the trees became more foliated, this dose was increased to 750 cc. The sand in each pot was leached fortnightly with tap water to prevent accumulation of salts or toxins.

Sixteen uniform and healthy 3-year-old trees of the Delicious variety were chosen from the nursery row of the Missouri Agricultural Experiment Station orchard at Columbia. Previous to planting, the roots of all trees were pruned by the Stringfellow method; that is, all the laterals were removed and only the "carrot stumps" left. All adhering particles of dirt and organic matter were washed from the root stump before potting in 12-inch clay pots. The normal winter pruning was given each tree. The tops of the pots were covered with roofing paper to prevent the quartz sand from becoming contaminated.

The trees were placed in a suitable area in the orchard, where they remained until samples were taken. Observations of the growth characteristics of each plant were taken at frequent intervals, special attention being paid to the foliage for possible development of leaf scorch. After five months' growth in quartz sand the trees were sampled for chemical analysis of leaves, 1-year wood, and 2-year wood. The analyses were made on an air-dry basis.

NITROGEN AND POTASH ANALYSIS

For the nitrogen, the official Kjeldahl-Gunning-Arnold method to include nitrate nitrogen was used. Analyses were made for both total and water-soluble nitrogen. Insoluble nitrogen was determined

by difference. For potassium, the official chloroplatinate method was used, analysis being made for total potassium only.

CARBOHYDRATE ANALYSIS

Analyses were made for total sugars, starch, and hemicelluloses. The total sugar was determined by thoroughly washing the sample with cold water and then clearing the filtrate with lead acetate. After inversion, the reducing power of the sugars was determined by the Shaffer and Hartman method. The starch was determined from the residue of the original sample by first gelatinizing the starch and then digesting it in freshly collected saliva at 38° C. After hydrolyzing with HCl (s.g. 1.125) for 2 hours, the reducing power of the resulting sugars was determined by the same method and calculated as glucose. The hemicellulose was determined by hydrolyzing the original residue of the starch determination with hydrochloric acid (s.g. 1.125) for 2 hours. The filtrate was then titrated in the same manner as for starch.

Results

The foliage characters exhibited by the plants of the different series were very characteristic, and were consonant with observations recorded by WALLACE (14). Leaf scorch symptoms did not appear in any series until after 2.5 months' growth. The incipient stages were characterized by a browning of the leaf apex, or sometimes small brown patches would appear along its margin. These brown dead areas gradually extended inward toward the midrib, but were not preceded by any noticeable chlorotic condition. Although enlargement of these scorched areas was very slow it was definite, and in the majority of cases started from the tip of the leaf.

The trees of series C were the first to exhibit the initial stages of leaf scorch. Its appearance was noticed after 10 weeks' growth, at which time noticeable shoot growth had stopped and terminal buds had formed. A few weeks later leaf scorch appeared in series E, but in this case most of the scorching occurred at the margin of the leaf instead of at the tip as in most other cases. By the fifteenth week all the trees seemed to have at least a few leaves whose tips had turned brown to a noticeable extent, but series C, E, and F developed the most severe cases. Toward the end of the experiment

the foliage of series A began to show a bronzed appearance, accompanied by brown or reddish brown spots dotted over the leaf area. The leaves of series B and D became pale yellowish-green in color and were rather sickly in appearance during the latter part of the growing period. Although several leaves turned brown at the apex, no severely scorched ones were observed. Series H developed leaf scorch to about the same extent as series A, but no badly affected leaves were found. Table I gives the data recorded at the time the samples were collected for chemical analyses.

TABLE I

SERIES	NO OF LEAVES SEVERELY SCORCHED	NO OF LEAVES SCORCHED AT TIP ONLY	NO OF NORMAL LEAVES	TOTAL
A	11	25	68	104
B	15	20	55	90
C	26	121	0	147
D	0	4	83	87
E	47	88	31	166
F	6	63	27	96
G	0	4	72	76

The observations recorded on each series of trees in these experiments seem to show clearly the effect of the N/K ratio of the nutrient media on the foliage of apple trees. It is well known that leaf scorch is a symptom of potash starvation (1, 8, 14), and this is verified in the present experiments by the results obtained in series C, which developed a severe case of leaf scorch. By the addition of a small amount of potassium (series F), leaf scorch developed on the foliage, but to a lesser degree than in series C. The addition of a greater amount of potassium (series G) again greatly reduced the amount of affected foliage. The addition of an excess of potassium practically eliminated all scorching tendencies. Series E, which was supplied with an excess of nitrogen, was also found to develop a severe case of leaf scorch. When this amount of nitrogen was reduced to one-third (series D), practically no scorch was developed. The results of these foliage observations seem to indicate that development of leaf scorch on apple trees is favored by a wide N/K ratio in the nutrient media.

The data for the chemical analyses of the leaves, 1- and 2-year-old wood, are given in tables II, III, and IV. Table II shows the

TABLE II
MACROCHEMICAL ANALYSIS OF APPLE LEAVES

SERIES	TREATMENT*	PERCENTAGE					
		Dry weight	Total nitrogen	Total potassium	Total sugars	Starch	Hemicelluloses
B .	0 ppm N	49.8	1.52	0.89	3.98	2.86	17.80
D .	36 ppm N	64.2	1.57	1.87	3.65	3.38	15.77
A .	110 ppm N	50.0	1.45	1.10	4.27	3.57	17.80
E .	323 ppm N	50.7	1.73	1.49	4.63	2.69	17.88
C .	0 ppm K	46.7	1.38	0.94	3.63	3.27	17.71
F .	45 ppm K	50.0	1.69	1.37	4.84	3.01	17.27
A .	126 ppm K	50.0	1.45	1.10	4.27	3.57	17.80
G .	368 ppm K	47.8	1.50	3.04	4.84	3.35	15.77

* Series B, D, A, E, potassium constant 126 ppm; series C, F, A, G, nitrogen constant 110 ppm

TABLE III
MACROCHEMICAL ANALYSIS OF 1-YEAR-OLD WOOD

SERIES	TREATMENT*	PERCENTAGE					
		Dry weight	Total nitrogen	Total potassium	Total sugars	Starch	Hemicelluloses
B .	0 ppm N	54.1	.77	.38	. . .	6.70	28.01
D .	37 ppm N	55.5	.85	.67	. . .	6.04	33.70
A . . .	110 ppm N	54.1	.69	.45	1.22	6.62	26.16
E . . .	323 ppm N	52.4	.81	.45	2.39	6.47	18.23
C . . .	0 ppm K	54.5	.55	.41	2.71	7.13	26.58
F . . .	45 ppm K	53.7	.64	.41	2.25	7.45	25.67
A . . .	126 ppm K	54.1	.69	.45	1.22	6.62	26.16
G . . .	368 ppm K	55.4	.61	.70	2.71	6.55	24.05

* Series B, D, A, E, potassium constant 126 ppm; series C, F, A, G, nitrogen constant 110 ppm.

TABLE IV
MACROCHEMICAL ANALYSIS OF 2-YEAR-OLD WOOD

SERIES	TREATMENT*	PERCENTAGE					
		Dry weight	Total nitrogen	Total potassium	Total sugars	Starch	Hemicelluloses
B	0 ppm N	56.7	.39	.25	1.69	6.47	22.50
D	36 ppm N	58.2	.41	.27	1.09	5.51	23.53
A	110 ppm N	58.9	.47	.28	1.88	5.98	23.28
E	323 ppm N	56.2	.61	.34	1.88	4.50	23.93
C	0 ppm K	60.9	.40	.44	1.22	5.72	24.61
F	45 ppm K	61.1	.47	.25	2.05	4.20	24.67
A	126 ppm K	58.9	.47	.28	1.88	5.98	23.28
G	368 ppm K	58.1	.53	.48	2.25	5.98	25.96

* Series B, D, A, E, potassium constant 126 ppm; series C, F, A, G, nitrogen constant 110 ppm.

effect of various amounts of nitrogen and potassium on the nitrogen, potassium, and carbohydrate content of the leaves. These data have little significance when considered from the standpoint of the effect of various N/K ratios. None of the determinations show any constant relationship to the N/K ratio of their nutrient solution, with the possible exception of the nitrogen determinations. The nitrogen contents of the leaves of the two wide ratio series (series E and F) are very similar, as are also the nitrogen contents of the two narrow ratio series (series D and G). The potassium contents of the two wide ratio series are also similar, but those of the narrow ratio series are not, owing perhaps to the luxury feeding of the high potassium series (series G).

Table III shows the effect of various quantities of nitrogen and potassium on the nitrogen, potassium, and carbohydrate content of the 1-year-old wood. These data are much the same as those in table II in regard to significant relationships. The potassium contents of the wide ratio series are somewhat similar, as are those of the narrow ratio series, but the nitrogen contents vary considerably. The potassium content of the 1-year-old wood seems to increase with increments of potassium. The nitrogen content also increases with the increments of potassium until a large excess is added (series G), when the nitrogen content decreases.

The data for the 2-year wood likewise fail to show any definite relationship with the N/K ratio. In the several series in which the potassium remained constant, both the nitrogen and the potassium content increased with increments of nitrogen. In the series in which the nitrogen remained constant, its content was found to increase as the potassium was increased in the nutrient solutions. Under the same conditions the dry weight decreased as the potassium was increased. In the several series in which the nitrogen remained constant and the potash varied, the minus-potassium cultures (series C) contained the least amount of nitrogen in leaves, 1-year and 2-year wood. The amount present in each series is shown in table V.

The leaves of the one-third potassium series (series F) absorbed the most nitrogen. In the 1-year wood the full nutrient solution absorbed the most nitrogen. In the 2-year wood the "potassium $\times 3$ " cultures (series G) absorbed the most nitrogen. In the several

series in which the potassium remained constant and the nitrogen varied, the minus-nitrogen cultures (series B) contained the least potassium in all three tissues analyzed. The amount of potassium present in each series is given in table VI.

The leaves and 1-year wood of the one-third nitrogen cultures (series D) contained the most potassium, while for the 2-year wood, the "nitrogen $\times 3$ " cultures (series E) contained the most potassium. In the 2-year wood the amount of potassium present increased as the

TABLE V
PERCENTAGE TOTAL NITROGEN

SERIES	LEAVES	SERIES	1 YEAR WOOD	SERIES	2 YEAR WOOD
F	1 69	A	0 69	G	0 53
G	1 50	F	0 64	F	0 47
A	1 45	G	0 61	A	0 47
C	1 38	C	0 55	C	0 40

TABLE VI
PERCENTAGE TOTAL POTASSIUM

SERIES	LEAVES	SERIES	1-YEAR WOOD	SERIES	2 YEAR WOOD
D	1 87	D	0 67	E	0 34
E	1 49	E	0 45	A	0 28
A	1 10	A	0 45	D	0 27
B	0 89	B	0 38	B	0 25

nitrogen was increased. These latter results indicate that the greater the amount of nitrogen applied to the plants the greater the amount of potassium needed.

The percentages of total sugars, starch, and hemicellulose are found to fluctuate considerably in all portions of the plant regardless of the treatment. They show no constant relationship to the amounts of potassium added to the nutrient solution or to its N/K ratio.

Discussion

The experiments here recorded appear to indicate that in the absence of an adequate supply of potassium the trees were unable to support the area of foliage produced. This suggestion is sup-

ported by the fact that when production of large leaves is stimulated by the addition of nitrogen, leaf scorch, which reduces the area of living foliage, becomes more pronounced. Conversely, by reducing the nitrogen supply and consequently rendering the plant less vigorous vegetatively, the potassium supply appears to be adequate for the smaller leaves produced and leaf scorch does not appear. This example serves to indicate that apart from the actual presence of these essential elements, they must be present in certain proportions before normal development will proceed.

The production of leaf scorch under these conditions indicates that potassium has more than one important function in plant development. Many investigators have shown that potassium is directly or indirectly essential for cell division, and probably for synthesis of proteins in meristematic tissue. If potassium plays such an important rôle in the production of new tissues, it must have an equally important rôle to play in the support and maintenance of this tissue after it is produced, because the amount of living tissue is reduced when a deficiency of potassium occurs.

The fact that leaf tissue is the first to turn brown and die indicates that potassium plays an important part in the functions of the leaf, probably in photosynthesis. Aside from the investigations of GREGORY and RICHARDS (4) and BRIGGS (2), very little work has been done dealing directly with potassium in relation to photosynthesis. These workers have shown, by measurement of freed oxygen, that carbon-dioxide assimilation is definitely retarded by limitation of potassium. GREGORY and RICHARDS also found that, under the conditions of their experiments, minus-potassium plants had a higher and minus-nitrate plants a lower respiration rate than complete-nutrient plants. Such results help to confirm the preceding explanation of the production of leaf scorch.

The macrochemical determinations of nitrogen, potassium, and carbohydrates of the leaves and wood of scorched trees were made in order to determine if possible a relation between the N/K ratio and the carbohydrate content of the plant. Carbohydrates have frequently been found to accumulate in plants deficient in potassium (3, 9, 12); likewise, nitrogen has frequently been found to accumulate in plants deficient in potassium. This is shown by NIGHTIN-

GALE (11), who found an accumulation of inorganic nitrogen in tomato plants; and STOKLASA, as quoted by NIGHTINGALE (11), presents analytical data showing that the percentage of inorganic nitrogen is much higher in minus- than in plus-potassium beets. Further, THOMAS (13), working with the apple, found that absorption and assimilation of nitrates was increased by additions of potassium, as indicated by the increased utilization of stored carbohydrates by the plus-potassium trees.

The analytical data obtained in the present experiments do not indicate any definite effect of potassium treatment on the carbohydrate content of the plant. This might be explained by the fact that the trees analyzed had grown but one season in the quartz sand, and when potted they contained considerable amounts of stored carbohydrates and minerals which undoubtedly greatly influenced the subsequent growth and analytical determinations. Evidently the carbohydrate contents of the plants were not among the factors causing leaf scorch.

Since it has been shown that plants deficient in potassium usually have a high carbohydrate content, which is also accompanied by a high nitrate nitrogen content, it seems logical to expect potassium to play an important part in determining the carbohydrate-nitrogen ratio in plants, which has recently been shown to affect the vegetative and reproductive states. In experiments with the tomato, KRAUS and KRAYBILL (10) demonstrated that nitrogen and carbohydrates are used in rather definite ratios for vegetative growth. HARVEY (5) found that in Grimes apples a moderate reduction of the carbohydrate-nitrogen ratio was accompanied by an increase in the rate of shoot growth, and an increase in the ratio was accompanied by decreased shoot growth.

HOOKE (7) has shown the carbohydrate-nitrogen relation to be associated more specifically with the process of flower bud differentiation in the apple. His observations are corroborated in the main by HARVEY and MURNEEK (6). The carbohydrate-nitrogen relation has also been investigated in connection with fruitfulness, sex differentiation, fruit set, and development of roots from cuttings, and many interesting correlations have been found. It is possible that in the various stages of its activities, the particular condition of the

apple spur may be expressed by a more or less definite relation of carbohydrates to nitrogen.

The foregoing experiments, as well as the literature reviewed, offer evidence that potassium plays a most important rôle in the growth and development of plants, and in some ways has a close relation to nitrogen in the chemistry of the life processes. Although the present experiments have failed to produce any direct evidence of a definite relation between nitrogen and potassium, the results indicate that there is a relation which is important to the normal growth of apple trees.

This investigation is being continued by the writer at the Missouri Agricultural Experiment Station on a much more extensive scale, and it is hoped that the final results will verify and add conclusive data to the indications pointed out in this preliminary investigation.

Summary

1. Observations were made on the foliage of a series of Delicious apple trees fertilized with nutrient solutions containing various N/K ratios. When the amount of nitrogen was reduced to one-third of that in the complete-nutrient solution, the development of leaf scorch was retarded and the amount reduced below that of all other cultures receiving additional nitrogen.

2. Increasing the amount of potassium in the complete-nutrient solution was also effective in preventing development of leaf scorch, being just as effective as the reduction of nitrogen. Decreasing the amount of potash or increasing the amount of nitrogen in the nutrient solution was conducive to leaf scorch development.

3. Data on the potassium, nitrogen, and carbohydrate content of the leaves, 1-year wood, and 2-year wood are presented. An attempt was made to correlate the carbohydrate content of the plants with their N/K ratio, but no correlation was found.

4. In general, it may be said that the response of the plants to potassium deficiency seemed to vary with the nitrogen content.

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[Accepted for publication July 15, 1931]

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MEIOSIS IN *HYPERICUM PUNCTATUM*¹

CARL SHERMAN HOAR

(WITH PLATES III, IV)

The genus *Hypericum*, which in some classifications is listed in the family Hypericaceae and in others under the heading Guttiferae, contains about 200 species. It is found growing in the north temperate and subtropical regions, but has a few species reported from the southern hemisphere. In size, the members of the genus vary from tiny herbs to rather large shrubs. For several years the writer has been making a general cytological study of those species common to the New England states, but since *Hypericum punctatum* appears to be distinct in its meiotic behavior, it seems best to discuss it separately from the rest.

Up to the present time no great amount of research has been carried out upon the genus *Hypericum*. NIELSEN (17) worked out the chromosome count in several species. He reported that only a small amount of work had been done previous to his research. TISCHLER (27) reported on the chromosome count of only two species. TREUB (28) investigated *Garcinia treubii*, and found a haploid count of 24. COHEN-STUART (26) found the haploid count in *Thea sinensis*, a closely allied species, to be 15. SCHNARF (24) investigated the embryology of the genus, and simply noted a large number of chromosomes. He did not investigate in detail.

NIELSEN found the haploid count of the species investigated to be as follows: *Hypericum ascyron* 9, *H. androsaemum* 20, *H. hircinum* 20, *H. coris* 9, *H. polyphyllum* 9, *H. hirsutum* 9, *H. maculatum* 8, *H. acutum* 8, *H. montanum* 8, *H. tomentosum* 8, *H. perforatum* 16, *H. organifolium* 9, *H. orientale* 8, *H. rumelicum* 7, and *H. prolificum* 9. In *H. montanum* he noted in the resting nucleus one nucleolus and about 20-30 chromocenters. Since there are but eight chromosomes, he concludes that the chromocenters cannot be prochromosomes. He found no irregularities in either the heterotypic or the homeo-

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typic division. The chromosome counts were easily ascertained except in *H. perforatum*.

CHATTAWAY (3) worked on several species, with special reference to chromosome size in *Hypericum calycinum*, and noted haploid counts as follows: *H. humifusum* 8, *H. quadrangulum* 8, *H. pulchrum* 9, *H. calycinum* 10, and *H. elegans* 16. He noted also that the numbers suggest the lack of multiples found in the work of ROSENBERG (22, 23) and BABCOCK and CLAUSEN (1) on *Crepis*. He called attention especially to the varying size of component chromosomes of *H. calycinum*. In 200 cases he observed one large outstanding chromosome, and four classes (1, 3, 3, and 3) with regard to size. The chromosomes of the homeotypic division are not only smaller than those of the heterotypic, but are of an entirely different shape, being long, narrow, and much more curved.

In preparing the material for the present study, specimens were collected in the field and killed in Carnoy's solution, its penetration being aided by the use of a hand vacuum pump. Other killing solutions, such as chromacetic and Flemming's, were also used but were not found successful. The material was imbedded in celloidin (Malinckrodt's pyroxylin purified), using the mass method described by JEFFREY (11). The sections were cut and stained with Haidenhain's iron-alum haematoxylin, and eosin was used as a counter-stain.

Hypericum punctatum Lam. (*H. maculatum* Walt. not Crtz.) is a distinct species growing throughout the New England region and farther south and west. Its large, oblong, round-tipped leaves, which have many black and pellucid dots, are quite striking. In Williamstown, Massachusetts, and in Pownal, Vermont, it was found growing in damp thickets beside the road. Apparently abundant moisture is one requisite for healthy growth, since it was much less plentiful during the last dry summer than formerly. It is characterized by a large percentage of sterile pollen, and has proved to be perhaps the most interesting, from the cytological standpoint, of all the species of *Hypericum* in the New England region.

There is a strong resemblance in the process of the formation of pollen to that observed by CLELAND (4-7) and others in *Oenothera*. The early development of the pollen mother cells resembles closely the illustrations of CLELAND (5) for *Oenothera*. The open spireme is

evident in the early stages, with no indication of doubling of the threads. The second contraction occurs with the threads massed together in the center, and those toward the periphery thrown more or less into loops. Out of this mass emerge individual chromosomes which are connected end-to-end. Fig. 1 illustrates the nucleus just before the second contraction. In fig. 2 the chromosomes are just emerging from the general mass. In fig. 3 is seen an early stage where the chainlike condition, together with the nucleolus, is evident, but the whole is still somewhat massed together. Fig. 4 shows the nucleus more enlarged, the chain of chromosomes more open, and the nucleolus gone. Sixteen chromosomes can be counted distinctly. Apparently the chromosomes in this case form one continuous chain, although this is not always evident. Under normal conditions the chain appears joined in an unbroken ring. In no case were paired chromosomes observed. In fig. 5 the chromosomes are shown as the nuclear wall disappears. Here, and especially in fig. 6, the chromosomes are still in chains after the nuclear membrane has disappeared. Fig. 6 is of special interest, since sixteen chromosomes appear to form a complete ring in the shape of a figure 8. Figs. 7-9 illustrate the appearance of the chromosomes at late prophase and early metaphase. Fig. 8 is a somewhat diagrammatic representation of a metaphase stage showing only those chromosomes at the upper focus. The alternation of the chromosomes as they separate is clear. In figs. 7 and 9 the whole complement of chromosomes is shown. Either because of the fixing agent, or for natural reasons, the chromosomes are clumped closely together, and are not open as in *Oenothera*, and complete alternation is not so clear. CLELAND (6) noted irregularities in *Oenothera*, and similar irregularities are often evident in *Hypericum punctatum*. In fig. 11 two chromosomes on the lower side appear to be out of place. In fig. 12 a chain of chromosomes is evident passing off from the general mass into the next mother cell. Fig. 10 illustrates the chromosomes at early anaphase. The connecting threads, which are more persistent in *Oenothera*, apparently break early in *H. punctatum*. Fig. 15 is an upper view of a stage at about the same time as that shown in fig. 10. One of the upper sets of chromosomes has wandered to the left, but the drawing also shows that both sets are arranged in more or less of a circle. In fig. 14 the

two equatorial plates are shown at the time of the second division. It is evident that the chromosomes still have a more or less curved shape. In both plates the haploid count is clearly eight. Fig. 16 shows a similar stage, only here the count is seven in the lower plate and nine in the upper. In fig. 17 a like condition is illustrated, but in this a dark line is evident in the cytoplasm, as though the chromosome in passing over had left a thread behind it. In fig. 13, illustrating the late anaphase of the first division, a lagging chromosome is shown. This doubtless depicts the process of making the uneven counts shown in figs. 16 and 17. As shown in figs. 21, 22, and 25, however, the lagging chromosomes may be lost entirely from the two resulting chromosome complements. The irregularity represented in fig. 11 probably accounts for such a condition as that in fig. 13, and also for those just mentioned. Similar irregularities were noted by CLELAND (6) in *Oenothera*, and also uneven counts in the second division complements. In fig. 18, an illustration of interkinesis, a small nucleus is shown which doubtless arose from the lagging chromosome. Figs. 21, 22, and 25 show various stages of the second division. It will be noted that the lagging chromosome has apparently been dropped from the first spindle and is undergoing further division. In no case have extra pollen grains been observed, but at maturity there are sometimes diminutive sterile pollen grains, indicating that they may be formed. Fig. 24 shows the condition during early interkinesis. It will be noted that, while there is some anastomosing among the chromosomes, they do not lose their identity. In fig. 23, a late stage during interkinesis, splitting of the chromosomes is evident. Fig. 20 illustrates the metaphase stage of the second division, with the chromosomes arranged in the usual manner and unlike that of the first division. Fig. 19 is a case of cytomixis. This is common in the species under discussion, but its significance is uncertain. As already stated, about one-half of the pollen grains abort at maturity.

In the work on *Oenothera* there is usually no mention made of the condition in the female gametophyte. The writer was able to observe several stages in the megasporogenesis of *Hypericum punctatum*, and the situation there was found to be much the same as in microsporogenesis. Fig. 26 shows a stage during "diakinesis" in the mega-

spore mother cell. The chain of sixteen chromosomes, with the nucleolus still present, is clear and striking. Fig. 27 shows a stage comparable with that in fig. 6. The chromosomes, although somewhat crowded, are clearly end-to-end, and apparently in a continuous ring. In fig. 29 the chromosomes appear at the late prophase, and the chain condition is still present. In fig. 28 the metaphase is illustrated, and, although they are crowded, it is evident that there is a tendency for the alternating chromosomes to pass to opposite poles.

Fig. 31 shows the pollen tube with its two male nuclei and the egg at about the time of fertilization. The degenerating synergids are not shown, since they would more or less obscure the egg. Embryos are frequent (fig. 30), but there are many cases in the buds in which the megasporangium has aborted and apparently fertilization has failed to take place.

Discussion

The cytological study of *Hypericum punctatum* is extremely interesting, in view of the chain or ring formation at "diakinesis" and its relation to the much discussed question of para- and telo-synapsis, to the situation in *Oenothera*, and to hybridization. Chromosomes in chains, although not common, are now known in several groups of animals and plants. KING (12) reported chain formation in *Bufo*, but the chain broke up before the first metaphase. STOUT (25) observed that the chromosomes in *Carex* had a serial arrangement, although again there was no alternation at the metaphase.

The whole question has been carefully worked out in *Oenothera*. GATES (9) first noted ring formation in the pollen mother cells of *O. rubrinervis*. CLELAND, studying *O. franciscana* (4), noted in the "late heterotypic prophase stages [corresponding to diakinesis in most plants]: (1) a marked tendency on the part of the homologous chromosomes to become paired, a condition quite in contrast to that in most of the *Oenotheras* so far studied; (2) a constant and definite arrangement of chromosomes in the nucleus; and (3) the association of certain chromosomes end-to-end throughout the period, in such a way as to form a closed circle." Later he (5) reported on *O. franciscana sulfurea*, which is a hybrid between *O. biennis* and *O. franciscana*, and noted that the failure of all but one pair of homol-

ogous chromosomes to pair suggests "the possibility that *Oenothera franciscana sulfurea* is to a large extent heterozygous."

CLELAND has studied the meiosis of several other species of *Oenothera*, among which may be mentioned that of *O. biennis* and *O. biennis sulfurea* (6). In these cases he finds no pairing of chromosomes in either species. Their diploid count is fourteen. Two rings, one of eight and one of six, are present at "diakinesis." This condition persists until the early anaphase, when the alternating chromosomes pass to opposite poles. A small percentage of cells show irregularities. Sometimes adjacent chromosomes pass to the same pole. Ordinarily another pair keeps the usual haploid count of seven constant by passing to the opposite pole, but sometimes a chromosome passes to the wrong pole, thus upsetting the count. Out of 500 cells studied he found that about 5 per cent showed unequal distribution. In any case, "irregularity in zigzag arrangement will probably have an important genetical result." He emphasizes two important facts: (1) "the presence of telosynapsis," and (2) "the irregularity that characterizes the whole process of meiosis in the pollen mother cells." He argues that since in only three of the eleven species studied is there complete absence of pairing, and since there are all gradations of pairing up to the presence of seven pairs in *O. blanda* and *O. deserens*, there is a definite meaning to the situation. He believes pairing takes place when the chromosomes are relatively homozygous but fails when they are relatively heterozygous. Those forms in which pairing is common are relatively stable while the others often mutate. This failure of homologous chromosomes to pair is common to many hybrids, and suggests that the lack of pairing in many species of *Oenothera* and in hybrids may be due to similar causes. He thinks that perhaps it did not come through hybridization, but rather through the "gradual accumulation of gene mutations," and the process is "aided probably by the appearance of balanced lethal factors."

DARLINGTON (8) made special reference to ring formation in *Oenothera* and other genera, and has attempted to show that there is no such phenomenon as telosynapsis. It seems fitting to cite here some of the research upon which he bases his conclusions. NEWTON and DARLINGTON (16), working on *Tulipa* and *Hyacinthus*, showed

that at the metaphase only homologous parts of chromosomes conjugate, and not chromosome entities. BOEDIJN (2) described parasynapsis in diploid and tetraploid varieties of *Oenothera*. JANSSENS (10) noted that in *Mecostethus* conjugation takes place side by side at one end only of the homologous chromosomes. NEWTON (15), working with *Fritillaria*, noted that the point attachment of the two chromosomes, corresponding to JANSSENS' chiasma, is fixed at the metaphase by the position of the attachment constriction. DARLINGTON therefore thinks that the parasynaptic system is sufficiently elastic to give results of the most widely divergent appearance at the metaphase. He considers the method of pairing in *Oenothera* as essentially parasynaptic, as urged on specific grounds, genetical and cytological, by OEHLKERS (18, 19) and RENNER (20, 21). Moreover, *Tradescantia* shows ring formation which is considered to be the result of earlier side-by-side conjugation and association by terminal chiasmata. In *Rumex acetosella*, KIHARA (13) has shown that ring formation follows side-by-side conjugation. DARLINGTON (8) argues that recent work on polyploid *Hyacinthus*, as well as observations of strings and rings of chromosomes in polyploid *Avena*, *Prunus*, *Primula*, *Solanum*, and other genera, makes it probable that ring formation in *Rumex* is actually the result of side-by-side conjugation, at different points, of more than two chromosomes; and that this species is in fact polyploid. Finally he shows by diagrams how rings may have arisen in tetraploid and diploid species of *Oenothera* parasynaptically. He concludes by saying that "ring formation in *Oenothera* has been shown to be associated with three genetic peculiarities: first, formation of a single linkage within the ring; second, the occurrence of a homozygote-eliminating mechanism similar in effect to the balanced lethal system in *Drosophila*; third, an extremely heterozygous condition of the plant."

The question as to whether *Hypericum punctatum* is a natural hybrid or not is an interesting one, but is difficult to solve. Its chromosome behavior and morphological sterility are much the same as commonly found in natural hybrids. Its external appearance, however, is quite distinct from any other species of the New England region. If its cytological resemblance to *Oenothera* is of significance and we follow CLELAND's conclusion, the condition may

have been brought about by gene mutation. It appears to the writer that at the present time there is not enough data to be absolutely sure. KERNER and OLIVER (14) and others have reported years ago that natural hybrids are common in *Hypericum*.

Summary

1. Microsporogenesis of *Hypericum punctatum* is interesting for the following reasons: (a) The chromosomes fail to pair at "diakinesis," and instead become fastened end-to-end in a chain; (b) at the first metaphase the chromosomes tend to separate so that the alternating members pass to opposite poles; (c) often irregularities occur so that the chromosome complements, at the time of the second metaphase, instead of having the usual haploid number of eight have seven and nine; (d) during the first division, chromosomes may lag and be lost from the spindle; (e) extra nuclei may be formed; (f) at interkinesis the chromosomes split longitudinally in the usual manner, and separate at the second anaphase; (g) during second division the extra chromosomes may form their own spindle and divide; (h) sometimes small abortive pollen grains are present; (i) at maturity nearly one-half of the pollen grains are morphologically sterile.

2. Megasporogenesis shows the same tendency to chain formation and to alternation of chromosomes at the first metaphase as found in microsporogenesis.

3. Meiosis in general is very similar to that found by CLELAND and others in certain *Oenothera* species.

4. The question of parasynapsis versus telosynapsis is discussed.

5. The general cytological situation closely resembles that found in many natural hybrids, but external characteristics are different from those of any other species of the New England region.

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[Accepted for publication May 15, 1931]

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EXPLANATION OF PLATES III, IV

All drawings are of *Hypericum punctatum* Lam. Magnification of figures is 3800 times unless otherwise specified.

FIG. 1.—Pollen mother cell showing nucleus just before second contraction.

FIG. 2.—Pollen mother cell showing nucleus with chromosomes just emerging after second contraction.

FIG. 3.—Pollen mother cell showing early stage of "diakinesis"; chromosomes in chains and nucleolus still present.

FIG. 4.—Pollen mother cell with nucleus enlarged, nucleolus disappeared, and chain of chromosomes so opened that sixteen can be seen clearly.

FIG. 5.—Pollen mother cell soon after nuclear membrane has disappeared; chromosomes still show tendency to chain formation.

FIG. 6.—Pollen mother cell after nuclear membrane has disappeared (note that chromosomes apparently form circle in shape of figure 8).

FIG. 7.—Pollen mother cell at first metaphase showing alternation of adjacent chromosomes.

FIG. 8.—Somewhat diagrammatic illustration of first metaphase showing only those chromosomes at upper focus (note regular alternation of adjacent chromosomes).

FIG. 9.—Chromosomes at first metaphase of pollen mother cell showing tendency to alternate.

FIG. 10.—Pollen mother cell at early anaphase of first division; connecting threads breaking.

FIG. 11.—Pollen mother cell at first metaphase showing irregularity in arrangement of chromosomes.

FIG. 12.—Pollen mother cells at early first metaphase showing chain of chromosomes extending into adjacent mother cell.

FIG. 13.—Anaphase of first division of pollen mother cell showing lagging chromosome.

FIG. 14.—Pollen mother cell with chromosomes at equatorial plate of second division (note curved shape of chromosomes and haploid count of 8 in each plate).

FIG. 15.—Upper view of pollen mother cell showing two rings of chromosomes at early anaphase (one above the other).

FIG. 16.—Pollen mother cell similar to that illustrated in fig. 14 but with one complement having 7 chromosomes, the other 9.

FIG. 17.—Pollen mother cell similar to that illustrated in fig. 16 (note dark line in cytoplasm between plates).

FIG. 18.—Pollen mother cell at interkinesis showing extra nucleus.

FIG. 19.—Pollen mother cell showing cytomixis.

FIG. 20.—Metaphase of second division of pollen mother cell showing chromosomes split in usual manner and unlike first division.

FIGS. 21, 22.—Various stages in second division of pollen mother cell showing that chromosomes often are lost from first spindle and undergo further division.

FIG. 23.—Late stage in interkinesis of pollen mother cell showing chromosome splitting.

FIG. 24.—Pollen mother cell at early stage of interkinesis; chromosomes, although showing some anastomosing, still keep their identity.

FIG. 25.—Similar to figs. 21 and 22.

FIG. 26.—Megaspore mother cell at diakinesis showing chain of 16 chromosomes; nucleolus still present.

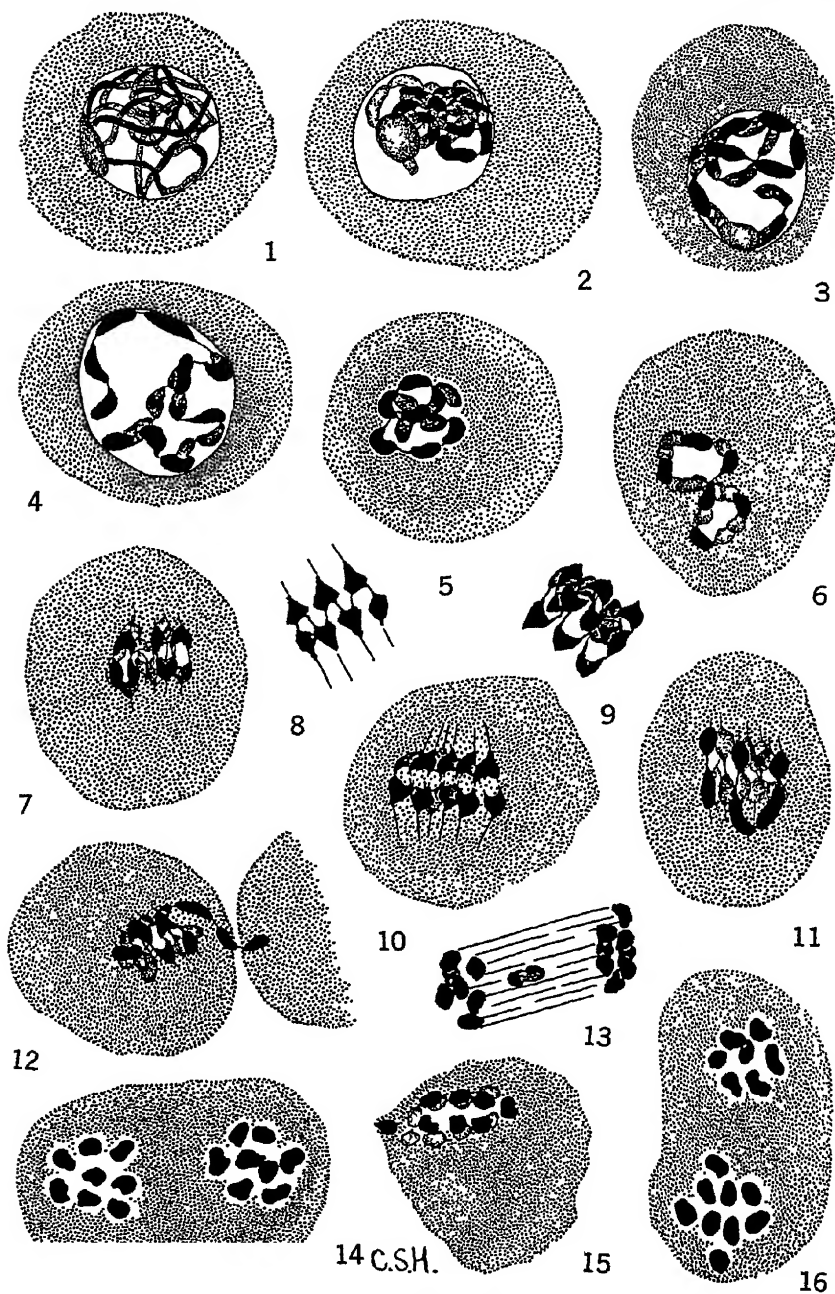
FIG. 27.—Megaspore mother cell at stage comparable with that shown in fig. 6; chromosomes, although overlapping, are clearly in chain.

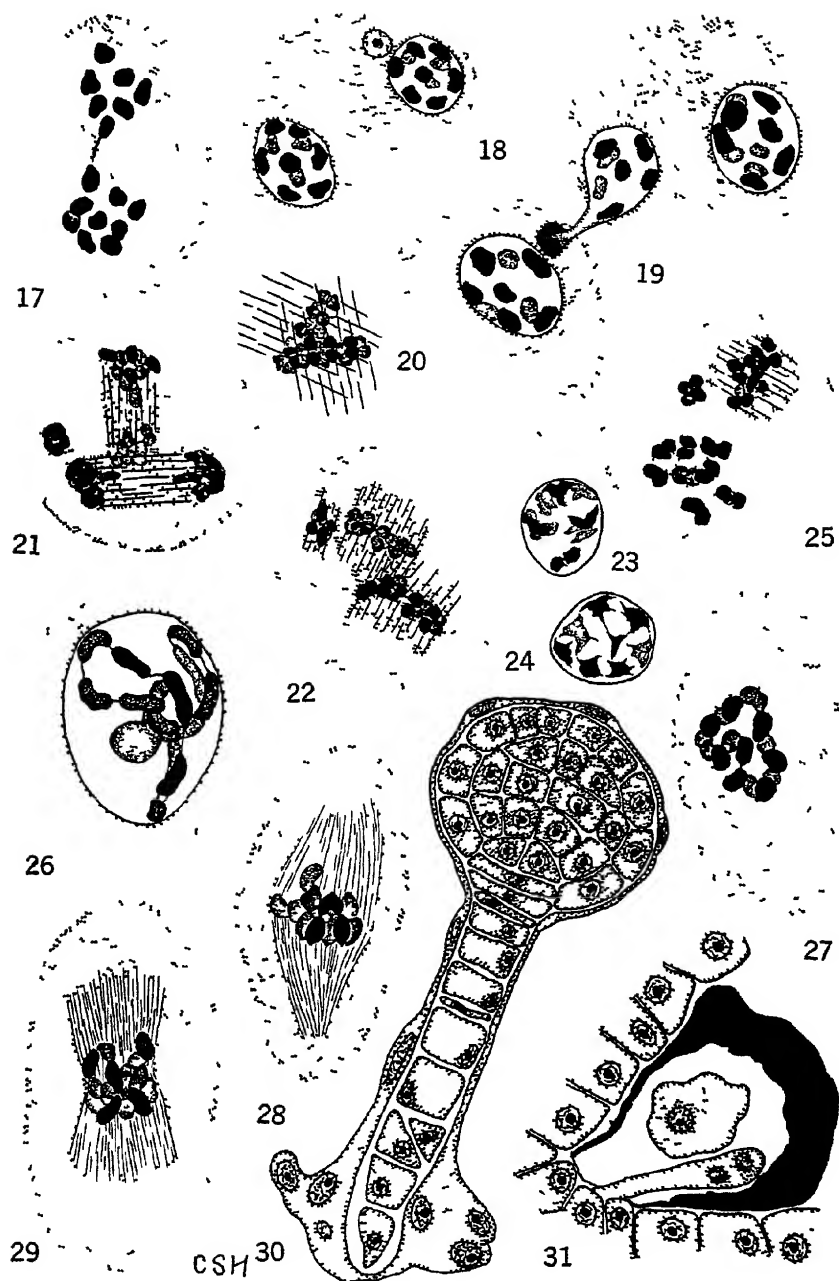
FIG. 28.—Megaspore mother cell at first metaphase; chromosomes, although crowded, show tendency to alternate.

FIG. 29.—Megaspore mother cell with chromosomes at late prophase.

FIG. 30.—Young embryo; $\times 900$.

FIG. 31.—Pollen tube about time of fertilization showing two male nuclei and egg; $\times 1200$.





ANATOMY OF THE PRIMARY AXIS OF SOLANUM MELONGENA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 426

ALBERT F. THIEL

(WITH TEN FIGURES)

Introduction

The eggplant, *Solanum melongena*, is grown as a truck crop in Florida, Louisiana, California, Virginia, New Jersey, and a few northern states. Because of its increasing commercial value and its importance as a food, some interest has developed as to its anatomy. Although the anatomy of closely related species in the Solanaceae has been investigated rather completely in some cases, the writer was unable to find any literature on the anatomy of this plant.

HARTIG (7) was the first to discover the bicollateral condition in plants, and HANSTEIN (6) first reported internal phloem in the Solanaceae. Since the bicollateral condition was first reported, investigators have found the same condition in eighteen families. HOLROYD (9) calls attention to the fact that "This advanced developmental condition is rare in the primitive cotyledons or Incompletae (Apetalae); is more frequent in the Apopetalae (Polypetalae); and most frequent, as well as most perfectly evolved, in the Sympetalae."

GERARD (5) described the transition from root to stem in *Cucumis melo* and *Cucurbita maxima*. He concluded that the internal phloem is merely a part of the external that is reoriented on the inner face of the bundle. HERAIL (8) studied the comparative anatomy of the stem of many dicotyledons, and concluded that the Cucurbitaceae alone possess a true bicollateral bundle. SCOTT and BREBNER (12), in their studies on *Thladiantha dubia*, found that the internal phloem connects with the external in the medullary ray. They found that during transition from stem to root the internal phloem passes out and unites with the external phloem.

FISCHER (4), studying the hypocotyl of *Cucurbita pepo*, found that the inner phloem gradually died out, ending blindly below. LA-

MOUNETTE (11) found no communication between external and internal phloem in *C. maxima*, and concluded that the development of internal phloem is an abnormal formation due to the activity of certain pith cells. VON FABER (14) traced the development of the bundles in the stem of *C. pepo*, and concluded that the internal phloem arises very early at the growing point. He found that both inner and outer phloem existed before any vessels were formed, and that the sieve tubes of the inner phloem originated from the same procambial strand as the rest of the bundle. WORSDELL (15) investigated the medullary phloem in the Cucurbitaceae, and concluded that it represents "a vestigial structure, the remnant of a former system of medullary vascular bundles in which the xylem has disappeared."

Mrs. SMITH (13), studying the development of *Dionaea muscipula*, reports that there is no transition in the hypocotyl. She states that the bundles are inverted as they leave the cotyledons.

ARTSCHWAGER (1), in his work on the anatomy of the potato, studied the root-stem transition, and states: "In the change from the exarch to the endarch condition it is noticed first, that the two protoxylem groups of a diarch root begin to swing outward, one following a left, the other a right curve." At a point just below the cotyledons he found that the primary xylem groups, instead of forming a radial row, come to lie in a tangential plane. The change from the exarch to the endarch condition was completed in the region above the cotyledons. With reference to the origin of the internal phloem, he concluded that it belongs to the stele proper and does not represent the vestigial remains of a secondary set of bundles. He found the first change in the primary phloem to be a breaking up of the two phloem groups with the formation of three or four smaller groups. These smaller groups orient themselves in such a way that two or three of them come to lie in the center of the stem, between the separating xylem strands. The other phloem groups take a position at the periphery of the stele.

Miss KING (10) traced the course of the bundles from root to stem in *Lycopersicum esculentum*, and found that the primary xylem plate differentiates into two distinct bundles. The metaxylem differentiates tangentially toward the periphery of the stele at successively higher levels. She found that the protoxylem maintained its

original position until the level just below the cotyledonary plate was reached. Centripetal differentiation of the protoxylem began at this point and continued until the primary xylem groups were almost endarch at the cotyledonary plate. With respect to the primary phloem, she found that it divided into smaller groups, and that strands differentiating toward the center constituted the internal phloem. The external phloem formed four equally distributed groups, which were oriented collaterally at the outer face of the xylem groups. Simultaneously the internal phloem formed two groups located at the inner limits of the primary xylem, thus establishing the bicollateral condition.

The investigations on the potato by ARTSCHWAGER, and on the tomato by Miss KING, show some variation in the method of transition in closely related species. Neither of these workers investigated the possibility of changes occurring in the petiole and midrib of the cotyledon. It was the purpose of this study to investigate the ontogeny of the root, the root-stem transition in the axis, and the vascular anatomy of the cotyledons.

Material and methods

The seed used is commonly known as the Black Beauty variety. BAILEY (2) classifies this variety as *Solanum melongena* L. var. *depressum* Bailey. The plants were grown in the greenhouse, and seedlings of different ages were used in the investigation. The 5-day-old seedlings furnished the best material for the study of root-stem transition, because at this age the primary body was fully differentiated and there was little or no development of secondary thickening due to cambial activity. Several killing agents were used, but the best results were obtained with Flemming's weaker solution. The usual laboratory methods of dehydrating and imbedding in paraffin were followed. The sections were cut to a thickness varying from $7\ \mu$ in the young root to $15\ \mu$ in the hypocotyl, and were stained with the triple stain of safranin, gentian violet, and orange.

Gross morphology

The ovules of *Solanum melongena* are campylotropous, and the cotyledons within the seed coats are erect with reference to the axis of the embryo. When proper conditions are provided, active germi-

nation begins in about four days. As a result of the absorption of water, the seed coat is split near the micropylar end. The hypocotyl emerges first through the split end, turning downward and differentiating the primary root. By further growth the hypocotyl partially withdraws the cotyledons from the seed coat, forming an arch, which straightens out by differential growth after it emerges from the soil. The seed coat adheres to the cotyledons for a few days, eventually falling to the ground. The 5-day-old seedling has a primary root approximately 4 cm. long, a hypocotyl 2 cm. long, and two cotyledons 1 cm. in length. The hypocotyl grows more rapidly than the epicotyl in the early stages of development, and the cotyledons are therefore the main photosynthetic organs during this early period.

The Black Beauty variety is a small and straggling plant, many of the branches finally resting on the ground. The pubescent leaves are alternate, simple, lobed, oblong, oval or ovate, unequal at the base, acute at the apex, and varying from 2 to 6 inches in length.

Ontogeny of root

Studies on the development of the primary structures were made with young roots 3 cm. in length. Transverse and longitudinal sections were made from the growing point to the region of maturation of the primary tissues. At the growing point the entire axis is promeristem. The cells are essentially isodiametric and alike; their walls are thin and without pits. The nuclei of the cells are large and the protoplasm is extremely dense, while vacuoles and intercellular spaces are absent. The primary meristems originating from the promeristem are not sharply marked. At about 5 mm. from the growing point, the regions of the stele, cortex, and epidermis are clearly distinguishable. The epidermal and cortical cells are clearly delimited, while most of the cells of the stele are still undifferentiated. Two glands are noticeable in the region of the pericycle. They appear before any protoxylem or protophloem cells are differentiated, and predesignate the location of these elements. They lie in the pericycle opposite the protophloem cells, and may be similar to the "sap-passages" mentioned by DEBARY (3) in the pericycle of primary tissues of the roots of the Umbelliferae. At a higher level, as the primary tissues reach maturation, these glands seem to be absorbed.

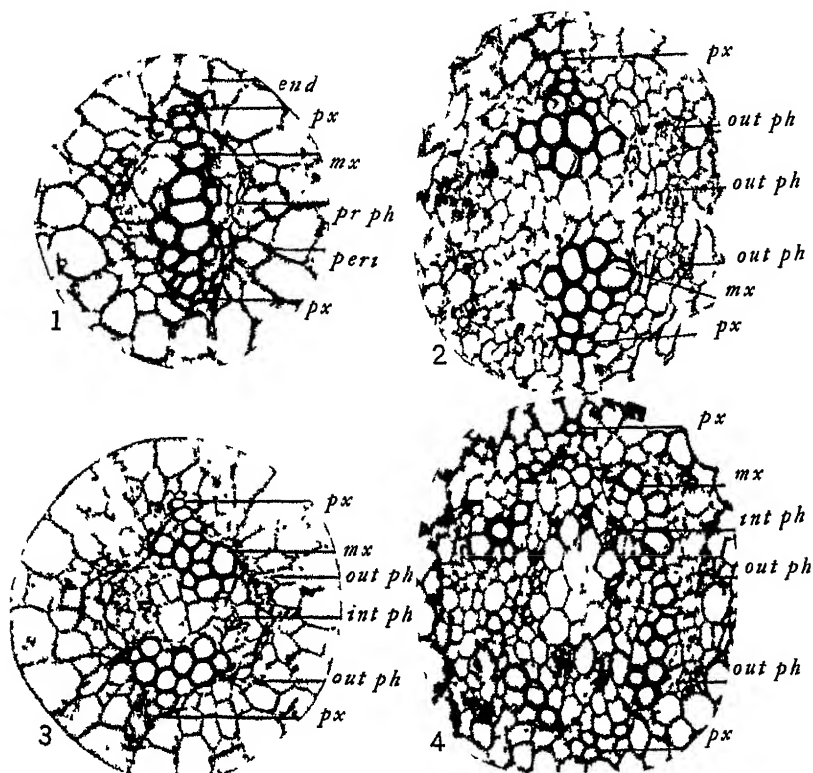
The epidermis and cortex are the first to differentiate from the primary meristems. The epidermis consists of a single layer of cells, greater in their vertical than in their radial dimension. Root hairs are numerous just beyond the region of elongation. The cortex surrounds the stele, and consists of a layer of parenchyma six cells in thickness. Its cells are arranged loosely and are much longer in their vertical than in their radial diameter. Large intercellular spaces are noticeable. The endodermis is composed of one layer of cells and is the innermost layer of the cortex. Its cells are somewhat longer, as seen in longitudinal section, than those of the pericycle, and their vertical dimension much exceeds the radial. The Casparian strips can be recognized at about 1 cm. above the root tip. The pericycle consists of a single layer of parenchymatous cells, and lies between the endodermis and the primary phloem. The protoxylem abuts directly against it. The cells of the pericycle are also longer in their vertical than in their radial diameter. Lateral roots originate from the pericycle cells, either opposite or at a slight tangent to the protoxylem points. A few of these were first observed at a time approximately coincident with maturation of the primary xylem elements.

The stele is a diarch, radial protostele (fig. 1). The protoxylem and protophloem are differentiated from the procambium simultaneously, development in both cases being exarch. The protoxylem is composed of long, slender, annular and spiral tracheae. The first vessels of the metaxylem to appear are the scalariform tracheae, and these are followed by reticulated and pitted tracheae. Parenchymatous cells separate the primary phloem groups from the diarch primary xylem plate. The protophloem is composed of elongated parenchymatous cells; the metaphloem, of sieve tubes, companion cells, and phloem parenchyma.

Root-stem transition

In studying the changes involved in passing from a diarch, radial protostele of the root to the bicollateral type of bundles in the cotyledons and stem, 5-day-old seedlings were used. At this age the hypocotyl was 2 cm. long and the cotyledons 1 cm. in length. In order to be certain of the exact location of the protoxylem points in the various sections with reference to the divergence of the cotyledons, whole

seedlings were used. By following this method the protoxylem points were always oriented in the same position on the slides, and less diffi-



FIGS. 1-4.—Fig. 1, transverse section of root showing primary tissues of stele, $\times 510$; fig. 2, transverse section of hypocotyl showing breaking up of diarch xylem plate and primary phloem groups, $\times 460$; fig. 3, same at somewhat higher level, showing beginning of bifurcation of metaxylem and inward differentiation of phloem, $\times 400$; fig. 4, transverse section of hypocotyl showing complete bifurcation of metaxylem and numerous internal phloem groups, $\times 490$.

*Abbreviations for all figures: *end*, endodermis; *epi*, epidermis; *gr pt*, growing point of stem axis; *int ph*, internal phloem; *mx*, metaxylem; *out ph*, outer phloem; *peri*, pericycle; *pr ph*, primary phloem; *px*, protoxylem.

culty was experienced in following the changes taking place in the serial sections.

The first indication of a change from the exarch condition begins very low in the hypocotyl, occurring approximately 2.25 cm. below

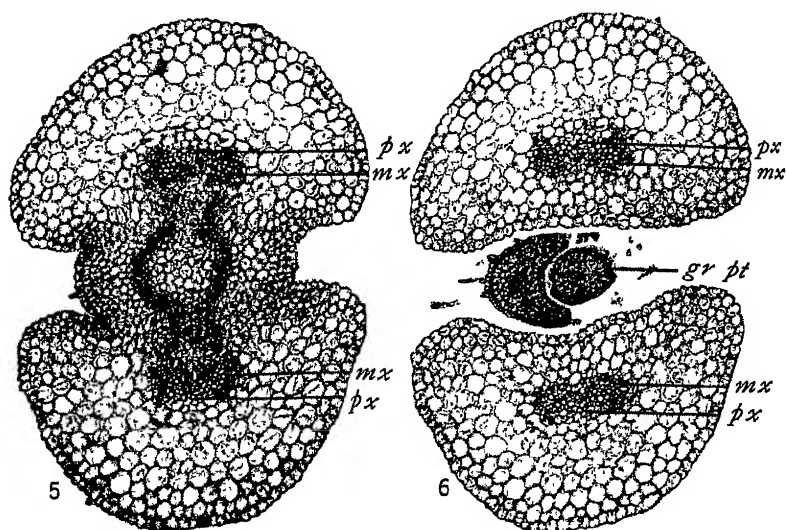
the cotyledons. All investigators to date report transition to be completed, either in the hypocotyl or stem axis; but in *Solanum melongena* it is completed in the bundles of the midrib of the cotyledons. The first change noted is a breaking up of the diarch xylem plate, forming two units of the primary xylem elements (figs. 1, 2). The cells in the central portion of the stele fail to differentiate as tracheae but remain parenchymatous. Simultaneously the two primary phloem groups each divide into three distinct groups (fig. 2). There is no indication at this level of an internal phloem. At a slightly higher level there is a bifurcation of the metaxylem of the two primary xylem units (figs. 3, 4). At this level there is a lateral rather than a centripetal differentiation of the metaxylem. The central group of phloem cells shown in fig. 2 are nearer to the center of the axis at the level shown in fig. 3. This inward differentiation from both sides continues until there are numerous small groups formed by further division of the original phloem groups (fig. 4). Each of the four remaining groups of the primary phloem lying nearest to the metaxylem are gradually inclined in a tangential direction toward the position of the protoxylem points.

At the level just below the cotyledonary plate the position of the protoxylem remains unchanged. The metaxylem of the two units has taken a position close to the periphery of the stele. The phloem groups are placed on both sides of the metaxylem, so that four tangentially oriented groups of primary xylem are differentiated, forming four bicollateral transition bundles. It should be noted, however, that at this level there is no outer phloem lying directly outside of the protoxylem elements.

Near the cotyledonary plate there is a gradual separation of the two double bundles formed by the breaking of the original diarch xylem plate and the reorientation of the phloem and metaxylem just described. One of these double units, consisting of one protoxylem point and its metaxylem, together with the internal and outer phloem groups, becomes the vascular trace of one of the cotyledons; and the second unit, that of the other. The protoxylem points have begun to change their position just below the cotyledonary plate, and at successively higher levels there is a gradual centripetal differentiation of the protoxylem and a centrifugal differentiation of the

metaxylem; but the endarch condition is not finally attained in the hypocotyl (fig. 5).

At the cotyledonary plate the direction of development of the primary xylem is still tangentially exarch (fig. 5), and there are numerous groups of internal phloem on the inner faces of the protoxylem and metaxylem elements. The outer phloem, however, is present only on the outer faces of the metaxylem groups (fig. 5). At a slightly

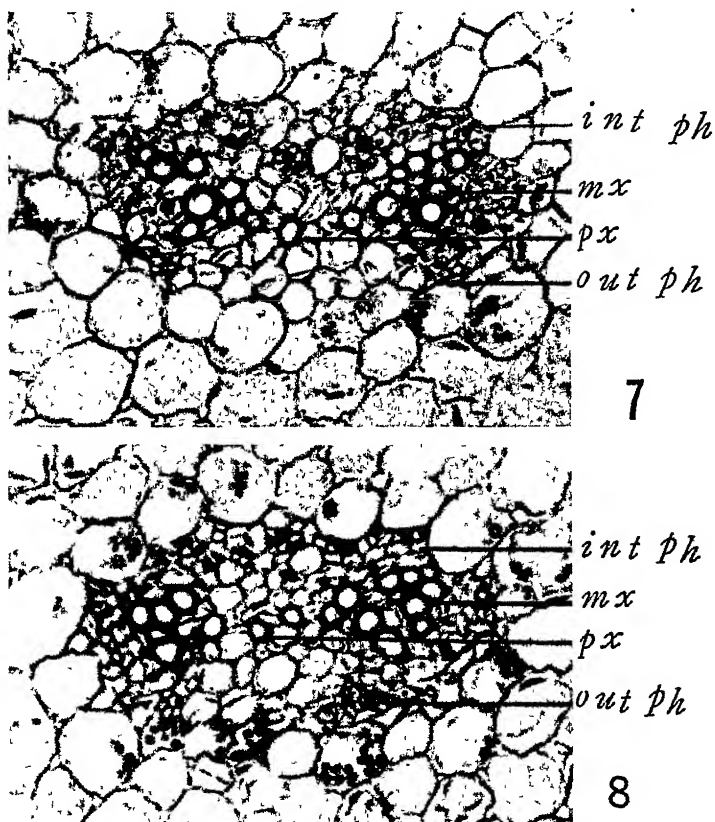


FIGS. 5, 6.—Fig. 5, transverse section through cotyledonary plate, showing complete separation of two units of original diarch xylem plate, each unit consisting of protoxylem with its bifurcated metaxylem together with internal and outer phloem groups, $\times 150$; fig. 6, transverse section through petiole of cotyledons 1 mm. above cotyledonary plate; primary xylem elements still exarch.

higher level, where there is complete divergence of the cotyledons, the same situation obtains with respect to the phloem in the cotyledonary petioles (fig. 6). The protoxylem and metaxylem are shifting their position; however, the former is developing adaxially with reference to the upper surface of the petiole, and the latter, abaxially.

The transition beyond the cotyledonary plate was followed in the petiole and midrib of one of the cotyledons (the illustrations are oriented so that the adaxial surface of the petiole and lamina are toward the top of the page). At successively higher levels the change

from the exarch to the endarch condition is accomplished gradually (figs. 7-10). The protoxylem differentiates adaxially, and finally assumes a position nearer to the upper epidermis than the metaxylem-

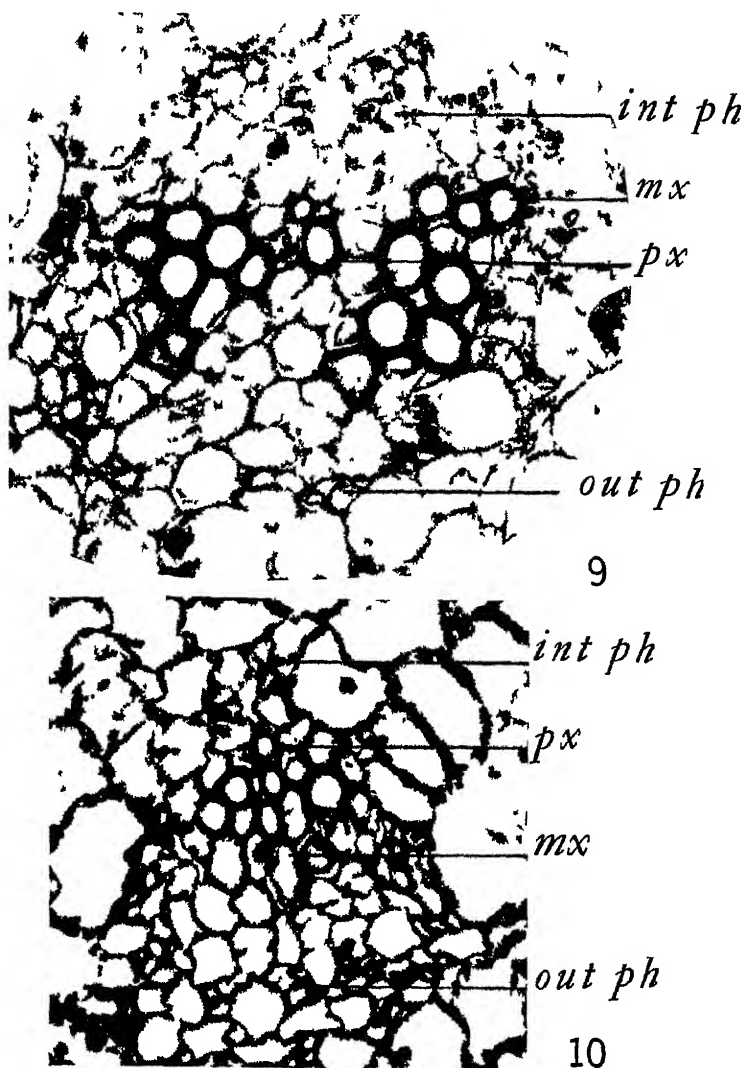


FIGS. 7, 8.—Transverse sections through petiole and lamina of cotyledon at successively higher levels; protoxylem differentiates adaxially and metaxylem abaxially until endarch condition is established (adaxial surface of petiole and lamina toward top of page).

lem. At the same time the metaxylem differentiates abaxially until the endarch condition is completely established (figs. 9, 10).

The internal phloem groups are numerous in the hypocotyl and petiole of the cotyledon. These groups gradually decrease in number in the bundle of the midrib until only one group remains opposite

the inner face of the protoxylem (figs. 9, 10). The outer phloem, lying opposite the outer faces of the two metaxylem groups, differ-



FIGS. 9, 10—Transverse sections through petiolar bundle at still higher levels in which endarch condition is established (adaxial surface of petiole and lamina toward top of page).

entiate toward the position originally occupied by the protoxylem point. These outer phloem groups are best illustrated in fig. 9. It will be noted that they incline progressively nearer one another until there is complete union of the two groups. The bicollateral condition is thus established.

The foliar traces in the first internode are completely endarch, and differentiate against the vascular elements of the hypocotyl slightly below the cotyledonary plate

Discussion

The method of root-stem transition in *Solanum melongena* agrees in general with the findings of Miss KING for the tomato. She did not follow the transition into the cotyledon, but found it almost completed at the cotyledonary plate. At this level in *S. melongena* the development of the primary xylem is still tangentially exarch. Her findings with respect to the behavior of the primary phloem groups in the tomato agree with the results of the writer. In working with the potato, ARTSCHWAGER found that the two primary xylem groups begin to swing outward, "one following a left, the other a right curve." The writer found no suggestion of this method in *S. melongena*. Instead of the two primary xylem groups following different curves, there was a bifurcation of the metaxylem. ARTSCHWAGER's findings with respect to the phloem agree with those of the writer.

With respect to the origin of the internal phloem, the writer agrees with those investigators who believe that the internal phloem passes out and unites with the external phloem in the root.

Summary

1. In *Solanum melongena*, the primary meristems, calyptragen, dermatogen, plerome, and periblem originating from the promeristem are not clearly differentiated.
2. The epidermis consists of one layer of cells from which root hairs arise; the cortex consists of six layers of parenchyma cells; and the endodermis and pericycle each consist of one layer. Lateral roots originate from the cells of the pericycle.
3. The stele of the primary root is a diarch, radial protostele.

4. Root-stem transition begins very low in the hypocotyl. The first change noted is a breaking up of the diarch xylem plate and the two primary phloem groups, forming two units of the primary xylem and phloem. At a higher level there is a bifurcation of the metaxylem and an inward differentiation of two of the phloem groups.

5. Near the cotyledonary plate there is a separation of the two double bundles formed by the breaking of the original diarch xylem plate. One of these double units becomes the vascular trace of one of the cotyledons, and the second unit, that of the other.

6. By inward differentiation and further division, several small primary phloem groups finally come to lie opposite the inner faces of the primary xylem elements. The four remaining groups lying nearest to the metaxylem are gradually inclined in a tangential direction, eventually lying on the outside of the original position of the protoxylem points. This development continues until the bicollateral condition is established.

7. The endarch condition is not attained in the hypocotyl but in the midrib of the cotyledon.

8. The foliar traces in the first internode are completely endarch, and differentiate against the vascular elements of the hypocotyl slightly below the cotyledonary plate.

Grateful acknowledgment is made to Dr. H. E. HAYWARD for valuable suggestions and criticisms during the progress of this work.

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[Accepted for publication May 22, 1931]

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PENETRATION OF SEED COATS BY ULTRA-VIOLET RADIATION¹

CHARLES A. SHULL AND HARVEY B. LEMON

(WITH PLATES V, VI)

Introduction

The importance of radiations of various wave lengths to plant and animal organisms has been recognized for many years. During the early period of investigation of radiation effects, attention was centered mainly upon the infra-red and visible radiations, which were known to affect profoundly the metabolism of organisms, particularly plants.

During more recent years, with the development of means of producing ultraviolet radiation and X-rays easily, great interest has been manifested in the destructive and the stimulatory effects of these shorter electromagnetic waves. With the discovery that phosphorus and calcium metabolism of animals can be modified by direct irradiation of the body, or even by feeding irradiated foods, and that radiations of certain wave lengths can be used to increase the rate of mutations in animals and plants, the physiological significance of radiations of all kinds has captivated the imagination, not only of biologists and physicists, but of people generally.

The relative ease with which short ultraviolet radiations are absorbed is well known. The value of these rays as photochemical reagents depends upon this ready absorption. The opacity of materials generally to the shortest ultraviolet has made it extremely difficult to study the effects of these radiations. Even gases and liquids quite transparent to visible light absorb the shortest waves; and the limit of solar ultraviolet seems to be set by the absorption of the rays by certain gases in the upper regions of the atmosphere, notably ozone.

In a general way, two physiologically different regions of ultraviolet have been recognized, the abiotic or destructive region, and

¹ This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

the biotic or biologically valuable region. The abiotic rays are the shorter ones, and the biotic rays are the longer. To ordinary plants the ultraviolet of sunlight is not harmful, nor does it seem to be useful, since plants have been grown very successfully without it (10). But some types of bacteria are killed by exposure to direct illumination. It must be clear that in order to determine the physiological effects of the ultraviolet radiations, one should have control of both the range of wave lengths and the intensity of the radiations. The common practice of using the open arc of the carbon lamp, or the complete range of the mercury arc, is open to grave criticisms. In such sources the possibly stimulative rays and the destructive rays are present together, and the harmful radiations usually mask the physiological effects of the longer rays. Under these circumstances one usually finds that the ultraviolet is harmful, or at least not beneficial. But screening out the abiotic rays, and controlling the intensity of the biotic rays, or separating them into individual bands, should make possible some real advance in the use of these radiations for modifying the metabolism of organisms.

Of just as great importance as the separation of the rays and control of intensity is the problem of the depth of penetration of the various regions of the ultraviolet spectrum. Most investigations have been made without the slightest knowledge of the penetrability of the materials, and with the general assumption that absorption occurs in the superficial layers of cell walls or protoplasm, and that deep-seated effects are not to be expected. While it has been easy to determine the range of wave lengths produced by the ultraviolet source, it is more difficult to determine the range of wave lengths which penetrate a given tissue, and to what depth each wave length penetrates.

Dry seeds have been treated with the mercury arc ultraviolet for as long as 188 hours without producing a noticeable effect on the subsequent germination of the seeds or on the growth of the seedlings after germination (9). Is this due to lack of sensitiveness of the protoplasm of the embryo in the dry, dormant state, or is it due to lack of penetration of the seed coats by the radiation? Just what radiations do pass through seed coats in the dry state, and how much more penetration occurs when they are wet? How much difference

is there in the penetrability of the coats of different types of seeds? And if the radiations do pass through the testas, how deeply do they penetrate the underlying tissues before they are completely absorbed? When a growing plant is irradiated, what range of wave lengths penetrates the cuticular covering of the cells? Which of the rays will pass through the entire leaf? If they penetrate the cuticle, but do not pass entirely through the leaf, just how deeply does each component of the wave-length range go? Correct answers to such questions are surely of fundamental importance, especially in the interpretations of results. No spectrographic records seem to have been made from seed coats, and no spectrograms of any kind have been found in the literature of plant physiology, although some studies have been made on leaves, as by DANGEARD (4), who describes his results, and states that certain leaves, *Tradescantia aurea*, *Pteris serratula*, *Selaginella kraussiana*, and *Panicum variegatum* would transmit rays down to 253 m μ ; others, like *Adiantum cuneatum*, *Begonia rex*, *B. crassicaulis*, and *Tradescantia zebrina* transmitted to 296-313 m μ ; and *Echeveria*, *Vriesea carinata*, and *Streptocarpus kewensis* showed a lower limit of penetration at 366 m μ . Even the visible rays at 404-435 m μ were transmitted but feebly in these plants. These are valuable data, and accurate measurements are needed on a wide variety of material.

Some recent studies on animal tissues indicated that certain of the ultraviolet radiations are more penetrative to skin, etc., than was previously thought. The earlier statements that penetration is limited to a depth of about 0.1 mm. were in some cases based upon the depth of injury. Since injuries are caused mainly by the very rays that are easily absorbed, it is obvious that this method of determining the penetrativeness of the ultraviolet reveals nothing about the penetrativeness of the biotic rays. These longer rays may penetrate much deeper without showing how deeply they have gone. The studies of MACHT, ANDERSON, and BELL (6) indicated that rays of 313 m μ could penetrate the entire body wall of the rabbit. This work was criticized by BACHEM (2), HILL (5), and PEARSON and GAIB (8); but in a more carefully controlled experiment, ANDERSON and MACHT (1) claim to have shown by fluorescent photometry, and by spectroscopy, that pieces of living skin 1.2 mm. thick were

penetrated by radiations of 253.7–300 m μ . They claim that penetration changed with death of the skin, dead skin being less easily penetrated than the living tissue. BACHEM and REED (3), however, think there is little difference between living and dead tissues if they are equally hydrated.

In view of the fact that statistical studies by MASURE (7), on germination of dry peas which had been treated with screened ultraviolet, indicated some influence upon subsequent germination, it was thought desirable to test the penetrability of a few seed coats. This paper presents the results of some preliminary tests, and does not attempt an exhaustive survey of the literature of ultraviolet penetration.

Materials and methods

The first attempts to demonstrate the presence or absence of penetration of ultraviolet were made with such resistant materials as mature potatoes, Jonathan apples, and leaves of *Bryophyllum calycinum*. Some immature potatoes with thin skins were used, also. The tests of seed coats involved the testas of peanuts, corn, peach, and cocklebur seeds. The corn and cocklebur seed coats are more or less translucent, while those of the peach and peanut are of heavier texture, more deeply pigmented, and more opaque to the visible rays. The results will probably apply to a rather wide range of material having seed coats of similar composition, texture, and density of pigmentation.

The potatoes, apples, and *Bryophyllum* leaves were exposed at a distance of about 20 cm. for 6 hours to the full range of the mercury arc from a Victor Uviarc laboratory outfit. The lamp was measured for its energy rate, which was found to be 105 watts. The intensity of the radiation cannot be stated in terms of candle-meter-second units, but a caesium-coated photoelectric cell indicated that the intensity of the radiations to which the cell was sensitive was approximately one-fifth as great when the G586AW screen was used as when the open arc was used. Individual lamps probably vary considerably in the intensity of radiations produced.

The spectrographic records reproduced in figs. 1–5 are all of penetration of seed coats. These spectrograms were made with an Adam Hilger type E3, which records the spectrum from 2100 to 7000 Å.

units. The photographic plates used were Wratten panchromatics, 10 inches in length, and the developer was bromided glycine.

The light source for figs. 1-4 was an Hanovia ultraviolet lamp. The seed coat membranes were removed from soaked seeds, trimmed carefully to avoid membrane defects, and dried flat with slight pressure. These membranes were placed in front of the slit of the spectrograph, and the radiations of the lamp focused upon the spectrograph slit by means of a simple quartz condenser. The exposure time was from 10 to 60 minutes. In some cases photographs were made with the membranes wet during exposure.

The photograph reproduced in fig. 5 was obtained by using the continuous ultraviolet spectrum of hydrogen. The source lamp in this case was a simple Geissler tube, filled with pure electrolytically produced hydrogen at a pressure of about 15 mm. One end of the Geissler tube is provided with a quartz window, through which the ultraviolet radiations were projected through the condensing lens upon the membrane. The hydrogen was rendered luminous by excitation from the secondary of an open core transformer, the primary of which was connected to 110-volt AC mains. The current was regulated by means of a rheostat. The exposures in this case were only one hour, and this was too short for the best results. It would probably require several hours for clear results with the hydrogen lamp. In many ways the hydrogen lamp is the more desirable source of radiations, since it permits one to determine the penetration of all wave lengths, and not merely of those bands which occur in the mercury spectrum. Also, if absorption bands occur in the ultraviolet range, the continuous spectrum would reveal the absorption, while the mercury spectrum might easily fail to show absorption bands. The main disadvantage is the long time period required for making good photographs with the hydrogen arc. To save time, the mercury arc was used in most of these tests.

Results

The experiments with potatoes, apples, and *Bryophyllum calycinum* leaves gave negative results. When they were treated with intact external structures, no injuries developed, either during or sub-

sequent to the exposure to six hours of radiation. Lack of injury may indicate that the shorter injurious rays did not pass through the cutinized or suberized surfaces, but there is nothing to indicate whether the longer harmless waves penetrated, or how deeply they may have gone.

Some of the potatoes were cut in two, and the cut surfaces exposed to the radiations. Eosin was used as a stain for detecting death of cells. These tests at the time were considered negative; but 24 hours after treatment, it was found that the exposed surfaces had turned black from the tyrosin-tyrosinase reaction which occurs in the dead cells of the potato. On cutting the pieces perpendicularly to the exposed surfaces, it was found that the blackened tissue had an average depth of 1 mm., and a maximum depth at one point of 3 mm. If death of the tissue in this case could be ascribed with certainty to the ultraviolet radiations, it would indicate penetration of harmful rays to a depth of 1-3 mm. The results give no information as to the penetration of the longer ultraviolet waves, and because of certain drying effects it cannot be said with certainty that the observed death of tissues below the cut surfaces was caused by the shorter ultraviolet, even though it may have been so caused.

It was then decided to make some spectrographic studies with membranes such as seed coats. Fig. 1 shows the results obtained with four specimens of seed coats from *Xanthium italicum*. The first and third spectra are of the mercury arc, exposed for 20 seconds. Between these two is a spectrogram of the first specimen, exposed for 45 minutes. Below the second arc spectrum are three others exposed for the same length of time. The last of these was kept wet during the exposure. The bands of the mercury spectrum between 3900 Å. (the limit of visibility) and 3630 Å. have penetrated the coats readily, and in the original plate there is some evidence of penetration to 3520 Å. All radiations shorter than this seem to have been absorbed by the coats. Wetting the coats made but little difference with the penetration range, although in some cases the intensity of penetration seemed to increase with the moisture content of the membranes. In fig. 1, however, there is little difference to be seen between no. 5, a dry specimen, and no. 6, which was wet.

In fig. 2 is presented a spectrogram made through the seed coats of the peanut. The only ultraviolet bands which show clearly in the photographs are those at 3620–3650 Å., although there is a faint record of the band at 3120 Å. in specimen no. 2. The Hanovia arc is from a 30-second exposure.

The most interesting record was obtained from corn grains, as shown in fig. 3. The Hanovia arc, 20-second exposure, is shown next to the scale. The first three spectrograms were taken through coats from the embryo side of the grain, and the last three through coats taken from the side opposite the embryos. Each exposure was for 10 minutes, and the third and sixth photographs were taken through wet coats. It is evident at once that the coats are more penetrable over the embryo face of the grain than over the reverse side. The coats from the embryo side of the grain have permitted all bands to penetrate to the 3630–3650 Å. region, with traces in the original plate of penetration at 3525 Å.; while the heavier coats from the backs of the grains absorb all radiations shorter than 3760 Å. In these cases wetting does not appear to have increased the penetrability of the coats.

The spectrograms shown in fig. 4 were made through peach seed coats. The first spectrogram next to the scale was taken with a dry coat, and with the vascular elements of the seed coat parallel to the spectrograph slit, while the second one was made with the vascular elements transverse to the slit. The third one was transverse also, but with the coat wet instead of dry. The exposure time for all of these was one hour. The arc itself is shown below, with 15 seconds' exposure. While the two dry specimens do not show penetration beyond about 3620 Å., the wet coat permitted radiations as short as 3020 Å. to penetrate feebly. In contrast to the results with corn seed coats, this is a clear case of moisture increasing the penetrability of the membranes.

The only record made with the continuous hydrogen spectrum is shown in fig. 5, which shows the results obtained with a 60-minute exposure with peanut coats. The first five tests were with dry coats, the last one with a wet coat. In the first specimen the radiations have penetrated to about 3030 Å., and in the sixth to about 3120 Å. The other records are too faint to show in the reproduction.

Discussion

The results, taken as a whole, show that seed coats allow only the longer ultraviolet radiations to penetrate. The lowest limit of transmission shown by any of the seed coats tested is at about 3020 \AA ., and penetration of rays shorter than 3630 \AA . is always feeble. Under these circumstances one would not expect ultraviolet radiation of seeds to produce injurious effects. On the other hand, the longer radiations do penetrate, and there is the possibility of stimulative action of irradiation such as MASURE (7) seems to have found in the case of peas. It should be remembered in this connection that the main ultraviolet bands transmitted by the G586AW screen used by MASURE are those at $3630\text{--}3650 \text{ \AA}$., which are the main bands transmitted by the seed coats.

The seed coats used in these tests are somewhat representative of seed coats in general, although none of the heavy coats, such as are found on some of the larger legumes, peas and beans, have been tested. It is not desirable to draw conclusions on any broader scale than the material used warrants. There is some variability in the penetration; and the case of membranes of the corn grain is interesting, showing as it does that different portions of the same seed coat are not alike in penetrability to radiations. These coats are usually considered to be non-living, and are therefore probably less penetrable in some cases than living membranes would be, on account of reduced hydration.

It is evident that such membranes as seed coats are to be looked upon as natural screens, with properties similar to the artificial gelatin and glass screens which are being used to restrict the range of wave lengths used in experimental work. All wave lengths longer than a certain minimum are able to penetrate, but the minimum varies with the individual membranes. Properly selected, one should be able to obtain a series of screens, of shorter and shorter range of the ultraviolet, among plant membranes. It seems doubtful whether any seed coats will transmit the rays shorter than the ultraviolet of sunlight.

It should be emphasized again that measurement of the depth, wave-length range, and intensity of penetration of radiations is fundamental to the interpretation of the results of such treatments.

Similar studies should be made of epidermal and other plant structures in connection with the study of beneficial and harmful effects of radiation of seedlings and of mature plants. Definite progress in this field in the future depends very largely upon the thoroughness with which the conditions of experimentation are analyzed. It is hoped that the results here presented may prove an incentive to more careful studies of penetration wherever radiations are being employed in biological research.

UNIVERSITY OF CHICAGO

[Accepted for publication May 9, 1931]

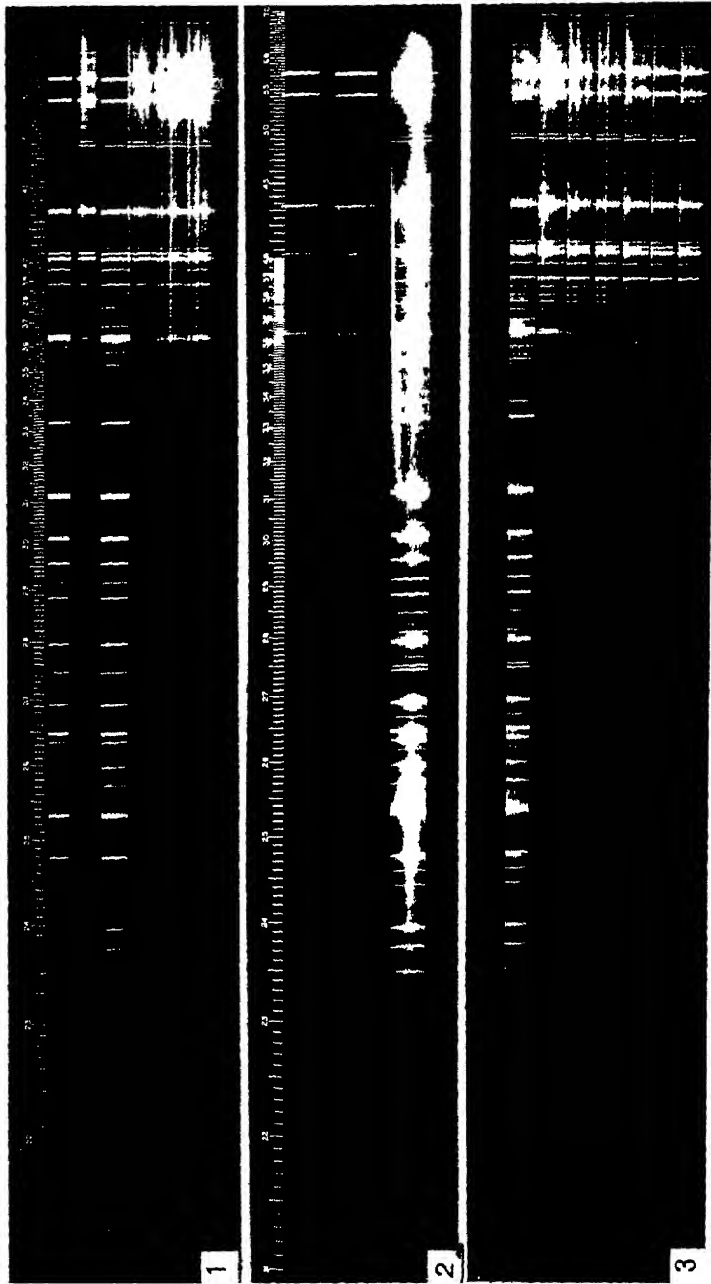
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EXPLANATIONS FOR PLATES V, VI

FIG. 1.—Spectrograms through coats of *Xanthium italicum*, slit 4, large diaphragm: no. 1, arc of Hanovia lamp, 20 seconds; no. 2, penetration of seed coat, 45 minutes, dry; no. 3, arc of lamp, 20 seconds; nos. 4, 5, penetration of seed coat, 45 minutes, dry; no. 6, penetration of seed coat, 45 minutes, wet.

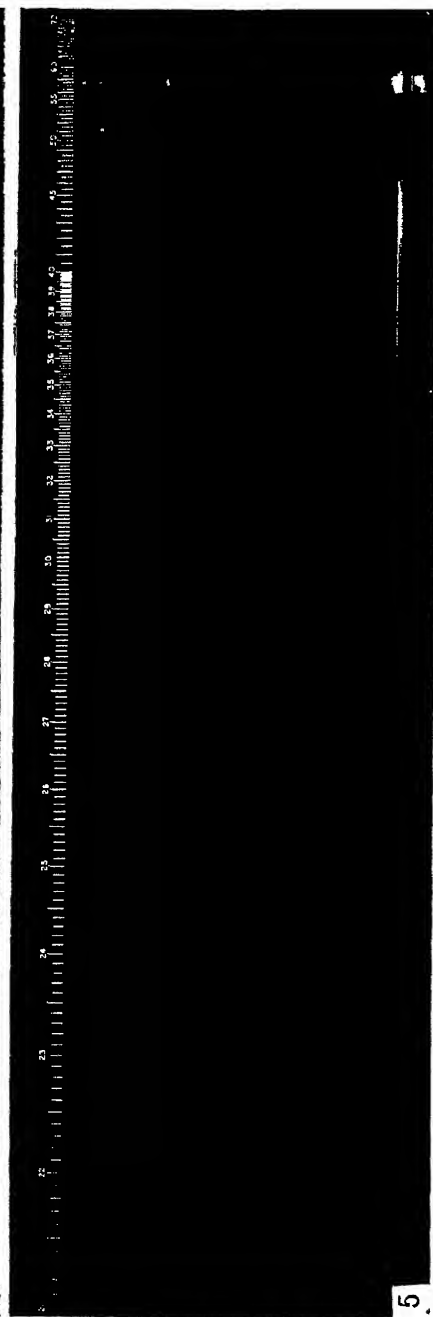
FIG. 2.—Spectrograms through seed coats of *Arachis hypogaea*, slit 4, large



SHULL & LEMON on ULTRAVIOLET PENETRATION



4



5

SHULL & LENON on ULTRAVIOLET PENETRATION

diaphragm: no. 1, penetration of seed coat, 60 minutes; no. 2, same, 60 minutes; no. 3, Hanovia arc, 30 seconds.

FIG. 3.—Spectrograms through seed coats of *Zea mays* (Reid's Yellow Dent), slit 4, large diaphragm: no. 1, Hanovia arc, 20 seconds; nos. 2, 3, 4, penetration of coats from embryo side of grains (nos. 2 and 3 dry, no. 4 wet), 10 minutes. Nos. 5, 6, 7, penetration of coats from back side of grain (nos. 5 and 6 dry, no. 7 wet); 10 minutes.

FIG. 4.—Spectrograms through coats of *Prunus persica* (Elberta), slit 4, diaphragm large, 60 minutes: no. 1, coat with vascular elements parallel to slit, dry; no. 2, coat with vascular elements crosswise of slit, dry; no. 3, same, wet; no. 4, Hanovia arc, 15 seconds.

FIG. 5.—Spectrograms through coats of *Arachis hypogaea* with hydrogen source, no diaphragm, 60 minutes: nos. 1, 2, 3, 4, 5, coats dry, no. 6, coat wet.

ACID INJURY OF COTTON ROOTS^{*}

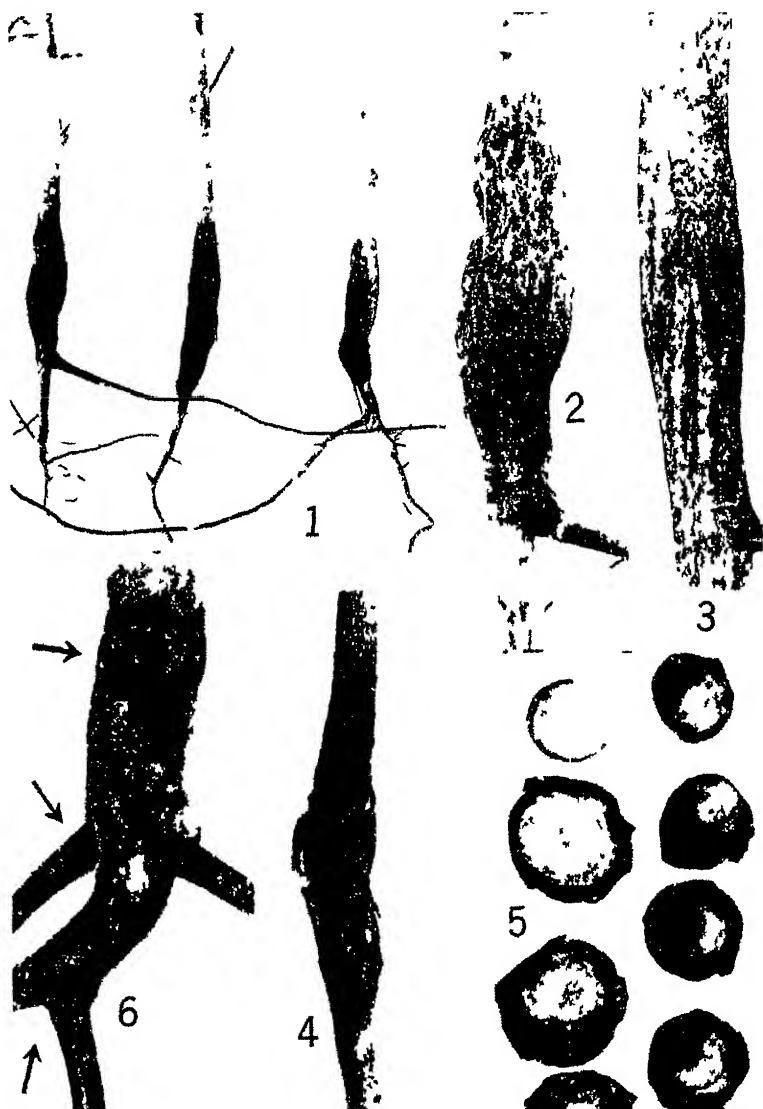
J. J. TAUBENHAUS AND WALTER N. EZEKILL

(WITH SIX FIGURES)

In an experiment on the relation of the soil reaction to cotton root-rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, containers of Lufkin fine sandy loam soil received surface applications of various materials mostly intended to change the soil toward acidity or alkalinity. The upper inch of soil was removed from each container, the materials worked into the second and third inches of subsurface soil, and the untreated surface soil then replaced. Cotton was planted in these containers and the plants were later inoculated with root-rot. Soil samples were taken periodically and the hydrogen-ion concentration determined colorimetrically. It was found that in series 11, in which sulphur had been applied at the rate of 5000 pounds per acre, the pH of the soil had changed appreciably, but that this change occurred only in the upper 2-8 inches of subsurface soil (table I), while the deeper soil remained at a pH of 7.3-7.6.

Although none of the plants in the containers of series 11 was attacked by root-rot, a new type of root injury was noticed, particularly when the cotton plants were pulled from all the containers for final examination at the end of the season. The upper parts of the tap roots were found to be peculiarly swollen and cracked (figs. 1-4), suggesting somewhat the effects of crown gall, while the roots of plants in the untreated soil in the check containers were normal except for plants infected with root-rot. Series of petri dish isolations were made, both from the injured and the sound tissues of the swollen roots from the sulphur-treated containers, but no growth appeared from any of the bits of tissue. The swelling and cracking of the cotton roots in the sulphur-treated soil was therefore probably due, not to any pathogenic organism, but rather directly to the excessive soil acidity brought about by the oxidation of the sulphur. This con-

^{*} Published with the approval of the Director as Contribution no. 157, Technical Series of the Texas Agricultural Experiment Station.



FIGS 1-6.—Roots of cotton plants with acid injury fig 1, general view showing location of affected areas, figs 2-4, enlarged views of affected areas, showing various amounts of cracking, fig 5, cross-sections through single affected root, showing extent of injury in different portions of injured area, fig. 6, slight injuries (indicated by arrows) on root taken from naturally acid soil in field.

clusion was supported further by the fact that the injury of the roots occurred in general at the same depth as the more highly acid soil layers (table I).

Acid injury from sulphur was observed again in an experiment in which various commercial sulphurs were applied to soil in small metal containers.² Sulphurs were applied in various series at 5000, 10,000, or 15,000 pounds per acre, after the containers were filled,

TABLE I
ACIDITY OF SOIL OF VARIOUS DEPTHS IN EXPERIMENTAL
CONTAINERS, REPRESENTATIVE OF SERIES 11, AS RE-
LATED TO LOCATION OF INJURED PORTIONS OF COTTON
ROOTS

CONTAINER	DEPTH IN INCHES	pH READINGS	INJURY TO ROOTS
11B	0-1	7.4	None
	1-2	4.3	Slight swelling
	2-3	3.5	Swollen and cracked
	3-4	3.0	Swollen and cracked
	4-5	3.0	Slight swelling
	5-6	3.0	None
	6-7	3.3	None
	7-8	4.9	None
11C	0-1	4.9	None
	1-2	3.7	Slight swelling
	2-3	3.0	Swollen and cracked
	3-4	3.0	Swollen and cracked
	4-5	3.1	Slight swelling
	5-6	4.3	None
	6-7	5.3	None
	7-8	5.0	None

and incorporated with only the surface 1 or 2 inches of soil. Cotton was planted and the plants inoculated with root-rot. At the end of the season all plants were pulled for examination of their roots. It was found that many of the dead plants had succumbed from acid injury, as evidenced by the characteristic enlarged cracked regions and the absence of *Phymatotrichum* strands or other symptoms of root-rot. Plants with acid injury were more abundant in the series with higher rates of application of sulphur than in those receiving 5000 pounds per acre; and, as shown in table II, were more abundant

² EZEKIEL, W. N., TAUBENHAUS, J. J., and CARLYLE, E. C., Soil-reaction effects on *Phymatotrichum* root-rot. *Phytopath.* 20:803-815. 1930.

in the series in which the soil actually became highly acid than in the series of less acid soils. Significant percentages of plants were killed by acid injury in the soils as acid as pH 3.0. Since the pH values for this series were obtained by colorimetric determination from soil samples which included the entire depth of soil in the containers, it is probable that the soil at the depth at which injury occurred was actually even more acid than indicated by the composite soil samples.

During 1927, and again during 1928, 1929, and 1930, acid injury to cotton roots was observed in sulphur-treated plats at College

TABLE II

ACID INJURY OBSERVED ON COTTON PLANTS GROWING IN SMALL CONTAINERS OF CROCKETT CLAY LOAM SOIL MATERIAL TO WHICH SULPHUR WAS ADDED, RESULTS ARRANGED BY AVERAGE pH OF SOIL WITHOUT REGARD TO TYPE AND AMOUNT OF SULPHUR

pH OF SOIL	NUMBER OF PLANTS	ACID INJURY OF PLANTS	
		Percentage injured	Percentage killed
2.1-2.5 .	41	75	17
2.6-3.0 . .	103	31	8
3.1-3.5 . . .	73	12	3
3.6-4.0 .	62	40	2
4.6-5.0	15	0	0
5.2-6.0 (checks) ..	45	0	0

Station and in various field experiments carried on by Mr. H. E. REA of Substation no. 5. The symptoms in all cases were the same as those originally found in the sulphur-treated containers during 1927.

A few cases of much less severe injury, although apparently of a similar type, have been found on cotton plants grown in the local Lufkin fine sandy loam soil without any artificial acidification. Localized, unusually large calluses were found on the tap roots of some plants growing in the more acid part of an experimental field (fig. 6); and dark, somewhat cracked, gall-like excrescences occurred on plants grown in containers of surface soil material, of pH 6.1. These swellings may have resulted from excessive callusing follow-

ing injury during cultivation, and apparently were not severe enough to interfere with good growth of the plants. In the experiment mentioned, injury of this sort did not occur in the plants in the four remaining series, in which soils with higher pH values were used.

DESCRIPTION OF INJURY.—Roots of cotton plants are sometimes injured by excessive acidity without noticeable symptoms appearing above ground. In more advanced cases, the affected plants die suddenly and thus might be mistaken for plants attacked by *Phymatotrichum* root-rot. When the roots are examined, however, the trouble is readily diagnosed. When surface applications of sulphur cause the injury, it is confined usually to a part of the tap root shortly below the surface of the ground. This region is swollen, and scarified by superficial longitudinal cracks in the bark, extending sometimes into the wood (figs. 3-5). The swelling, beginning an inch or two below the surface of the ground, may include 2-6 inches of the tap root, and occasionally may involve also small parts of one or two of the lateral roots (fig. 1). On well developed roots, the diameter of the swollen area is frequently twice that of the adjacent normal part of the root. With younger plants the swelling of the roots may be slight, but such plants are more likely to die from the injury than is the case with older plants. Occasionally the cracks in the swollen part of the tap root become so extensive that the top of the plant falls over and snaps off. Portions of the tap roots or lateral roots below the swollen areas usually appear normal, at least on the surface. Sectioning affected roots either longitudinally or transversely, portions of the vascular tissue in the injured area were found to be conspicuously reddened. During dry seasons, cotton plants growing in acid soils may die suddenly, the tap roots in the acid layers of soil turning red and darkening without the swelling and cracking that occurs during a wet season. This reddening accompanying acid injury is brighter and lighter in shade than the reddish brown discoloration of the tissues characteristic of *Phymatotrichum* root-rot.

It should be added that when cotton is planted in very acid soils, with a pH of 3.0-4.0, the seeds frequently fail to germinate or the seedlings die soon after germination. During wet weather the seed-

lings are usually attacked by various microorganisms, particularly *Fusarium* and *Trichoderma*.

Histological studies of normal and acid-injured cotton roots are reported in the paper by GORE and TAUBENHAUS³ included in this issue.

Summary

In experiments in which extreme soil acidities resulted from excessive application of sulphur, injury to cotton roots was evidenced by a characteristic enlargement and cracking which eventuated often in death of the plants. The injured areas of the tap roots were found to correspond to the location of the more acid layers of soil (at pH 2 to 4). A few less severe cases were found in the field and in experiments with a soil naturally at pH 6.1.

TEXAS AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS

[Accepted for publication July 13, 1931]

³ GORE, U. R., and TAUBENHAUS, J. J., Anatomy of normal and acid-injured cotton roots. BOT. GAZ. 92:436-441. 1931.

ANATOMY OF NORMAL AND ACID-INJURED COTTON ROOTS^{*}

U. R. GORE AND J. J. TAUBENHAUS

(WITH TEN FIGURES)

Introduction

This paper reports the results of anatomical studies of acid-injured cotton roots. In a preceding paper, TAUBENHAUS and EZEKIEL (6) described a type of injury of cotton roots growing in an acid soil. Externally such roots appear greatly swollen, with a much broken bark and deep longitudinal lesions.

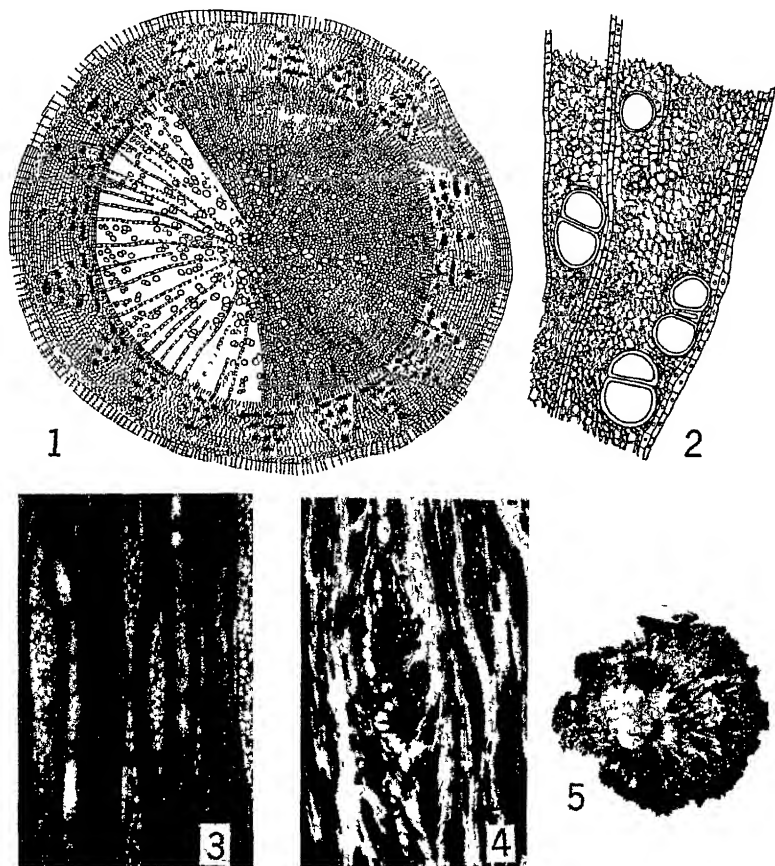
It is well known that proliferations and overgrowths in plants may be induced by chemical stimuli. SMITH (5) produced tumors on cauliflower leaves by treatment with formic acid, formaldehyde, acetic acid, and ammonia. These tumors consisted largely of hyperplasia and hypertrophy of the leaf with some killing of the cells. WALLACE (7, 8) found that ethylene gas may produce intumescences and proliferations on stems and buds of the apple. Similar results were obtained by HARVEY and ROSE (3) with low concentrations of ethylene gas on tomato and *Hibiscus* roots. LA RUE (2) reported intumescences on poplar leaves grown in air with a high carbon dioxide concentration.

MATERIAL.—The material used in these studies consisted of normal and proliferated mature cotton roots grown at College Station in field plats of Lufkin fine sandy loam soil, highly acidified by the surface application of sulphur. The degree of injury in the proliferated material studied varied from mild proliferations to extreme hypertrophy. The wounded tissue was taken well below the root-stem transition zone.

Sections were cut from fresh material on a sliding microtome. Owing to the large amount of woody tissue, this material could not be imbedded in paraffin with any degree of success. A combination

^{*} Published with the approval of the Director as Contribution no. 158, Technical Series of the Texas Agricultural Experiment Station.

stain of safranin and Delafield's haematoxylin with iron-alum was used in most cases.



FIGS. 1-5.*—Fig. 1, Semidiagrammatic drawing representing structure of normal cotton root; fig. 2, transverse section of portion of normal xylem; fig. 3, tangential section of normal xylem; fig. 4, tangential section of proliferated xylem; fig. 5, transverse section of extreme proliferation with isolated areas of secondary xylem.

*Photomicrographs made with 8 \times compensating ocular, 16 \times apochromatic objective and aplanatic condenser, and Leitz camera. Drawings made with aid of a microprojector.

Normal roots

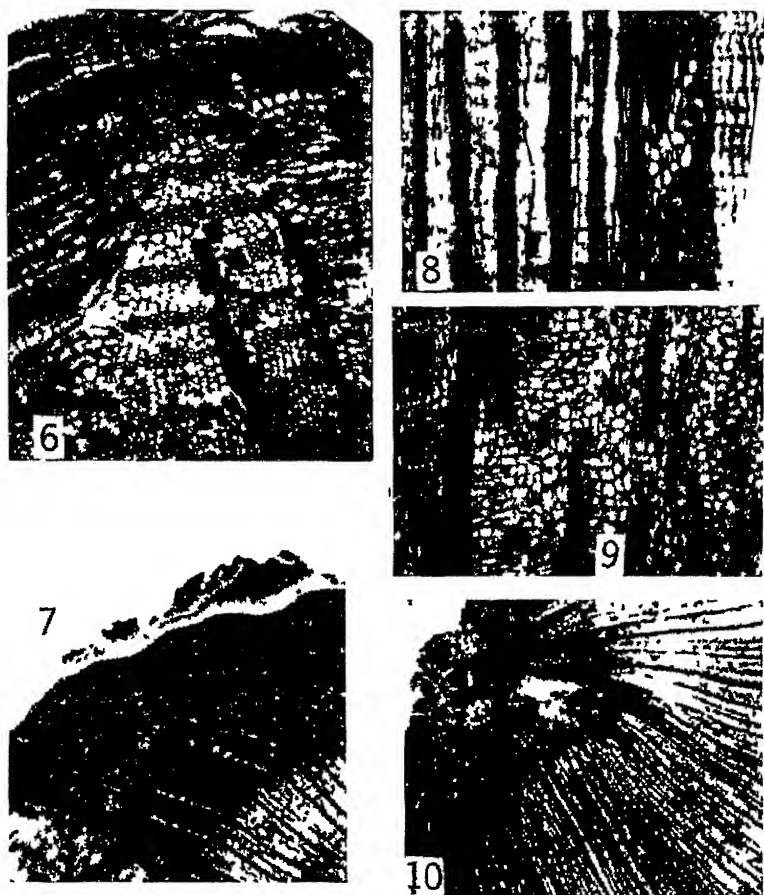
The general arrangement of the secondary tissues of a mature cotton root (fig. 1) bears a close resemblance to that of the basswood stem. In a mature normal cotton root the secondary xylem makes

up the greater part of the wood, the primary xylem occupying a small portion in the center of the axis. For convenience, the secondary tissues are considered separately under a longitudinal and a radial system. This classification has been used by EAMES and MAC DANIELS (1). In the xylem the longitudinal system consists of large pitted vessels and wood fibers; the radial system consists of rays only, which are composed of parenchyma cells. As seen in cross-section, the vessels are scattered irregularly throughout the xylem, occurring either singly or in groups of two to four (fig. 2). All the vessels occurring in the stele of a normal root are shown in fig. 1. In tangential section the vessel appears as a continuous tube, with vessel segments varying in form, but frequently about twice as long as wide (fig. 3). The cross walls may be horizontal or at a slight angle. The lumina of the vessels frequently contain conspicuous tyloses. The pits are of the bordered type. The fiber is typical wood fiber, with thick walls, tapering ends, and bordered pits. The xylem rays are one to four seriate and many tiers of cells deep (fig. 3).

The longitudinal system of the secondary phloem consists of regular areas of sieve tubes and companion cells alternating radially with groups of phloem fibers. The radial system consists of vascular rays, outside the cambium, which appear in transverse section greatly broadened in the outward direction, forming wedge-shaped areas. The two types of wedge-shaped areas alternate with each other tangentially, the rays filling the space intervening between the regions of the longitudinal system, as shown in fig. 6. Phloem rays, only a few cells in width, may subdivide these regions of the longitudinal system (fig. 6). This increase in the external portion of the phloem rays is the result of cell division, together with an increase in the tangential dimension of the cells. Widening of the outer portion of the phloem rays in this way allows for a uniform increase in diameter of the root without causing breaks in the phloem. A cork cambium arises in the outer region of the pericycle, and by repeated division it produces a periderm of the usual type, consisting of many layers of protective cork cells. Resin glands similar to those found in the stem, leaves, and seed, are found also in the phloem rays of the root.

Acid-injured roots

When cotton is grown in soil with a pH of 3.0-4.0, the cells of the vascular rays are stimulated and become meristematic. The cam-



FIGS 6-10.—Fig 6, transverse section of normal phloem; fig. 7, transverse section of proliferated phloem and periderm; fig. 8, radial section of normal phloem and periderm; fig. 9, radial section of proliferated phloem with resin glands, fig. 10, transverse section of proliferated rays with isolated area of primary xylem.

bium and the pericycle also become stimulated, with the result that they produce an abnormal amount of new tissue. The combined stimulation of these parts results in enormous hypertrophies of the

root. It is well known that such ground tissues as pith, medullary rays, cortex, and epidermis become meristematic under certain conditions, and KÜSTER (4) states that the epidermis proliferates least readily and lignified cells such as vessels and wood fibers do not produce new cells.

The nature of the proliferations in the cotton root was found to vary with the severity of the acid injury. In mild cases of injury only the phloem region is affected, while in more severe cases both xylem and phloem are involved. At first the original phloem is apparently killed and cut off from the inner tissues (fig. 7). These cells become compressed, forming a protective layer for the tissues underneath. That this brown corky layer is phloem can be recognized easily in cross as well as in longitudinal sections. Just underneath this layer of functionless phloem, several new layers of cork are produced, apparently by a new cork cambium. The cambium produces new phloem and new phloem rays which are regular in their arrangement. In severe cases all of these cells are parenchyma with few sieve tubes and phloem fibers (fig. 9). The division of the cork cambium also forms a layer of cork many times thicker than the cork of the normal root (fig. 7). The total thickness of the phloem is also greater.

In many instances of mild proliferation large masses of parenchyma cells have been found imbedded among the elements of normal xylem (fig. 10). These masses are formed by excessive division of the xylem rays. In extreme proliferations, areas of normal xylem are found to be completely separated by parenchyma formed in the same manner (fig. 5). In either mild or extreme proliferation, the cells of the cambium divide excessively and produce xylem and phloem containing variable quantities of the usual elements. The xylem and phloem are abnormal in that they may be several times their usual thickness.

Proliferated xylem in contrast to normal xylem shows shortened and distorted vessel segments, as well as a decrease in the number of vessels (fig. 4). Wood fibers are pushed out of their normal position. The proliferated cells of the xylem rays are thin walled and nearly isodiametric. The cells of the proliferated phloem rays are very similar, except that they are not quite so uniform. Tyloses are

often found in the vessels of the proliferated tissue, but apparently they are no more abundant than in normal tissue.

Summary

Marked proliferations of acid-injured cotton roots were found to arise from cell division of the ray cells of the xylem and phloem, and from a stimulation of the cambium and phellogen. The original phloem may be killed and become functionless. New phloem is produced which may contain the same elements although in varying proportions. The proliferated cells are generally thin walled parenchyma cells varying somewhat in size and form. Tyloses were present in both cases, but do not seem to be related to acid injury.

TEXAS AGRICULTURAL EXPERIMENT STATION
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[Accepted for publication July 13, 1931]

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STUDIES IN SUGAR CANE PHYSIOLOGY^{*}

II. SUGAR CANE GASES

AUGUSTO BONAZZI

(WITH TWO FIGURES)

It is a known fact that when a sugar cane is cut so as to expose the young reserve parenchymatous tissues to the air, these tissues soon change, first to a reddish color and later to dark brown to black. This discoloration is especially pronounced in the upper or younger portion of the cane.

During a study of the mechanism of this discoloration, and in connection with the localization of growth centers in the apical portion of the cane, it was found necessary to inquire into the composition of the gases which are found in these tissues. This paper summarizes some of the determinations made in this connection during the past few years.

Pieces of sugar cane, cut under water, and made of appropriate size, still under water, were introduced as soon as cut into a container (fig. 1*A*) filled with water and connected through a mercury pump (*B*) to a gas reservoir filled with washed mercury. By means of the mercury pump a vacuum was made in *A*, whereby all the gases contained in the tissues escaped as bubbles and collected in the neck of *A*, whence they were driven to the reservoir *C*. This operation was repeated until the tissues released no more gas or only a very insignificant quantity of it. Heat was used in expelling all traces of gas in the container *A*. The contents of *C* were then transferred to the gas burette *D* and submitted to analysis.

During the first part of this study, a burette holding 1.5 cc. of gas was used for the analysis; later, when the amount of tissue derived from the more basal portions of the cane was such as to yield greater quantities of gas, a burette was used which contained 11.5 cc. Alkaline pyrogallol and phosphorus were used at various times for the absorption of oxygen, while for the absorption of carbon dioxide

^{*} Contribution from the Chemistry Laboratory of the Estación Experimental Agronómica, Santiago de las Vegas, Cuba.

a solution containing 127 gm. of potassium hydroxide in 64 gm. of water was adopted. The accuracy of the apparatus and reagents was frequently checked with outside air. No determination was made for other gases.

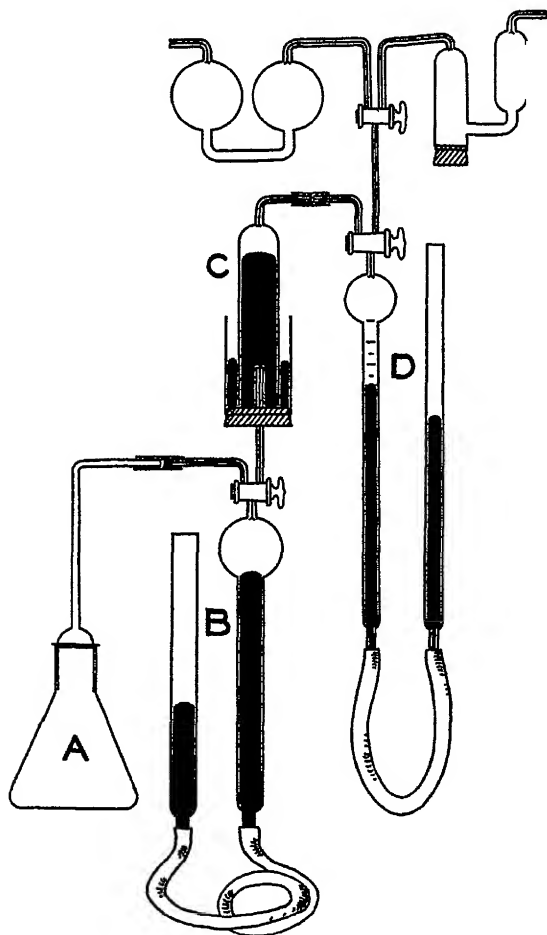


FIG 1

Table I summarizes the results obtained on direct analysis, corrected to 0° C. and 760 mm. barometric pressure.

Little needs to be added to what is already known relative to the gas changes induced by respiring plants in their gaseous environ-

ment. It is significant, however, that tissues of the plants themselves, in this case sugar cane, are subject to deep seated modifications in the percentage composition of the tissue gases. Recently GAERLAN,² studying the composition of the gases held in the inner chamber of bamboo stalks, found the air therein to have a relatively high carbon dioxide and a low oxygen content. The actual composition of the gaseous mixture varied with the time of day. In the

TABLE I
DIRECT ANALYSIS; TIME OF DAY, P M.

No of OBSERVATION	PORTION OF CANE WHENCE TISSUE WAS DERIVED	PERCENTAGE COMPOSITION	
		CO ₂	O ₂
1 .	Apical buds 3 cm. long	50 59	0 71
2 .		51 04	1 01
3		60 54	0 59
4		58.75	1 19
5	Apical buds and 5 internodes 12 cm. long	35 52	9 36
6	Median internodes 30 cm. long	17 23	19 71
7		17 44	16 48
8		17 72	12 78
9	Basal internodes 30 cm. long	8 07	13 14
10		7.39	15 07
Summary of results			
Apical buds		53 23	0.87
Subapical internodes		35.52	9 36
Median internodes		17 46	16.32
Basal internodes.. . . .		7.73	14.10

present study it was found that the relative abundance of oxygen in the tissues of sugar cane apparently varies inversely with growth capacity of the tissues themselves, moisture content of the bud tissues, and their cryoscopic coefficient.³ It is therefore apparent that these differences in germinative capacity of the tissues are closely connected with the differences in gaseous composition just mentioned.

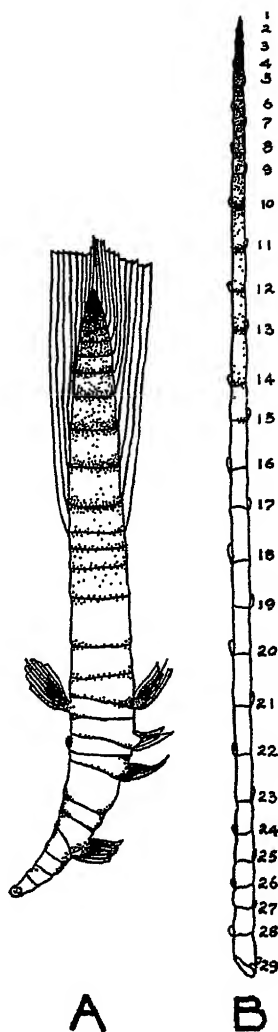
By splitting sugar canes longitudinally at different stages of maturity and flooding the cut surfaces with guayac tincture, it is possible to localize, in a rough way, the oxidizing enzymes. Invari-

² GAERLAN, S. A., Philip. Agric. 14:557-567. 1926.

³ BONAZZI, A., Planter and Sugar Manuf. 29: September. 1928.

ably a deep blue color, indicative of these enzymes, manifests itself in the apical tissues immediately after addition of the reagent, whereas only a faint blue coloration shows in the tissues of the median and basal portions, and that after a much longer lapse of time. This is illustrated in fig. 2 (stippling indicates guayac reaction), which shows a young developing cane sprout (*A*) still attached to its underground rhizome, with several buds in process of germination at its base; and a fully mature cane stalk (*B*) separated from its underground portion, with many resting buds in its median and basal portions. Evidently the younger the tissues the greater is the content of oxidases, the greater the consumption of oxygen (probably a high reduction potential), and the more abundant the elimination of carbon dioxide which, accumulating in the tissues, leads to the results indicated. The internal environment of the young growing tissue, therefore, is greatly different from that of the more mature portions, situated as these are in a medium much richer in oxygen.

It may be mentioned in passing that, as a result of these studies, it is evident that any investigation on the activities of parasitic forms (such as the sugar cane mosaic) which pass part if not the greater portion of their life cycle in the apical and rapidly growing portions of the plant, should be conducted, not only in a gaseous environment of low oxygen tension, but of a high carbon dioxide tension.



NEEDLE STRUCTURE AS AN AID IN DISTINGUISHING COLORADO BLUE SPRUCE
FROM ENGELMANN SPRUCE

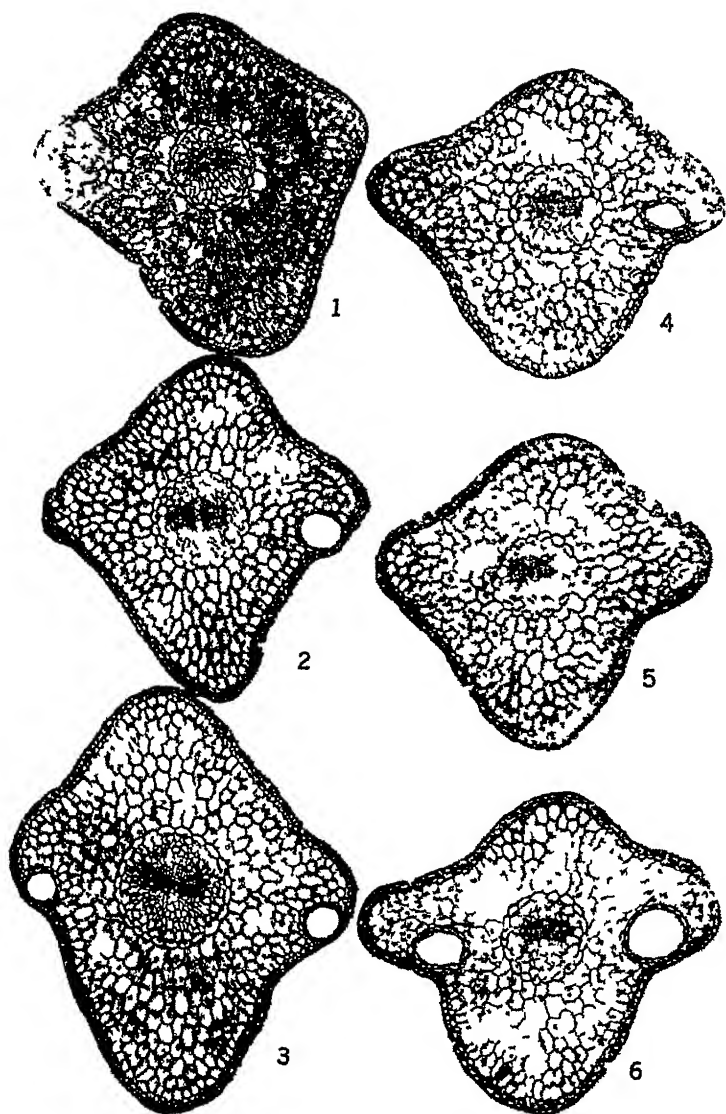
H I MARCO

(WITH SIX FIGURES)

Picea pungens Andre and *P. engelmannii* Engelmann are essentially Rocky Mountain species, growing in rather definite altitudinal ranges. Difficulty is often experienced in distinguishing between these two trees when they are found out of their natural habitat and also in that small zone where their ranges overlap. This difficulty is more pronounced in ornamental specimens. The needles in both species are awl-shaped or quadrangular in transverse section, and vary in color from dull grey-green or blue-green to silvery white, the latter being more characteristic of Colorado blue spruce. In both trees the needles extend from all sides of the twig, but in Engelmann spruce the angle of growth and the arching are such that a somewhat flattened spray results. In contrast, the needles of Colorado blue spruce bristle from all sides of the twig, are stiffer, and more keenly pointed than those of Engelmann spruce, in both species they are about 1-2 cm. in length. The twigs of Engelmann spruce are moderately stout and are usually darkly pubescent for the first two or three years, in comparison those of the other species are stout, rigid, and glabrous. These external features are often unreliable in young trees, and it is sometimes difficult to distinguish seedlings of the two species.

Transverse sections of the needles of both species have the following anatomical features in common: an epidermis of the usual type, consisting of a single row of cells, a hypoderm of a single layer which becomes two- to three-layered in the angles of the needle; a circular endodermis of cells approximately equal in size but variable in wall thickness,¹ and two fibrovascular bundles. Resin canals also

¹ The relative thickness of the inner and outer walls seems to vary somewhat with the locality, this is especially striking when material from the west is compared with eastern-grown stock.



FIGS 1-6.—Figs 1-3, cross sections of needles of Engelmann spruce 1, upper portion of needle showing no resin canals, 2, half way down from tip of needle showing one resin canal, 3, basal section showing two resin canals. Figs 4-6, cross-sections of needles of Colorado blue spruce 4, showing presence of one canal, 5, section half-way down from tip of needle showing absence of resin canals, 6, basal section with two resin canals

* Photographs made from slides stained with Bismarck brown (in 70 per cent alcohol)

occur externally in the mesophyll or green tissues of both species, and are of primary importance since they furnish a means of differentiation.

The results here reported are based on a study of approximately 90 needles of Engelmann and Colorado blue spruce respectively, taken from different places in the crown of several trees growing locally (Syracuse, New York), and also of a like number taken in a similar manner from two trees of each species at stations in central and southern Colorado.

SARGENT (1) by illustration, and SUDWORTH (2, 3) by description, indicate that but one resin canal is evident in the needles of Colorado blue spruce, and that this is located in one of the angles, while resin canals are wanting altogether in the leaves of Engelmann spruce. This study has shown that this statement is incorrect, since resin canals occur in both species. These are not of the usual type found so universally in pine needles, however, but consist of longitudinal series of short cavities (cysts), separated by partitions of mesophyll. It is therefore understandable why transverse sections taken at random through the needles of these species may fail to show canals. Figs. 1-6, showing transverse sections taken at different heights in the same needle, illustrate this situation. The vertical distribution of these "short" canals in the needles was followed by making serial sections 1 mm. apart from the base to the apex. Since canals were wanting in most instances in the upper half of Engelmann spruce needles, while one or more were invariably present in the Colorado blue spruce, this difference seems to offer a rather reliable means for distinguishing these two species, provided a number of needles are available for sampling.

In summation, the needles of both Engelmann and Colorado blue spruce contain "short" resin canals, arranged in longitudinal series; two such series occur in the basal half of the needles. In Colorado blue spruce these series of short canals extend into the upper half of the needle, while the same does not hold for Engelmann spruce.

The writer is indebted to Dr. H. P. BROWN and Dr. W. M. HARLOW for helpful suggestions in preparing this paper. The receipt of material is gratefully acknowledged from Chief Ranger J. S.

McLaughlin, of the Rocky Mountain National Park, Colo., and Mr. Elmon B. Radway, of Apulia, N. Y.

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[Accepted for publication June 8, 1931]

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A NEW SPECIES OF BIDENS FROM AFRICA

EARL EDWARD SHILIFF

Bidens praecox sp. nov.—Herba erecta, gracilis, glabra, erecte ramosa, circ. 6 dm. alta, caule plus minusve tetragona. Folia tenuiter petiolata petiolis saepe albido-hispidis ± 1 cm. longis, petiolo adjecto 4–7 cm. longa, 1–2-pinnata, segmentis ultimis valde membranaceis, nunc ovatis nunc rhomboideo-oblongis, breviter rotundodentatis et apicaliter rotundatis sed obsolete mucronulatis, acriter ciliatis, circ. 4–12 mm. latis. Capitula verisimiliter discoidea, ramulos (pedunculos) acriter angulatos glabratos vel irregulariter hispideos usque ad circ. 8 cm. longos terminantia, ad anthesin minuta (verisimiliter circ. 5 mm. alta et circ. 4 mm. lata). Involucri glabri bracteae exteriores circ. 8, tergo 3-nervatae, sursum sensim dilatatae, apicaliter subito rotundatae et obtusae vel rarius subacutae, eciliatae, demum etiam usque ad 4 mm. longae; interiores oblongolanceolatae apicaliter angustatae ac pulverulente pubescentes, demum circ. 8–9 mm. longae. Achaenia praecocia, tenuiter clavato-lineararia, obcompressa vel plana, deorsum sensim angustata, plumbeo-atra, glabra vel tuberculato-papillata, utraque facie nunc eleganter circ. 8-sulcata nunc subobsolete circ. 4-sulcata, 6–7.5 mm. longa et circ. 0.8–1.1 mm. lata, marginibus exalata, apice exaristata sed plus minusve incrassato-capitata vel crassiusculo-annulata, itaque sub apice plerumque plus minusve constricta, demum paleas oblongo-lineares hyalinas apicem acutum versus sensim angustatas dimidio superantia.

Specimens examined: *Dr. Walter Busse* 2523, in sandy soil, sunny places in legume forest, Mayanga, District of Lindi, southeasternmost German East Africa, May 15, 1903 (type in Herb. Berl.).

Florets both ray and disk are lacking on the type. The achenes had been well formed even on small, young heads, differing in this respect from those in ordinary species of *Bidens*. Moreover, the achenes are seen to have produced at their apex some sort of secondary growth, this imparting a more or less capitate appearance. The

growth in question is mostly 0.5–0.8 the maximum width of the achenial body, and is variously truncate or protuberant, ovoid or subglobose, sometimes appearing faintly as if representing a remnant of the disk floret. The general habit is that of many annual species of *Bidens*, to which genus the species is apparently best referred for the present.

CHICAGO NORMAL COLLEGE

CURRENT LITERATURE

BOOK REVIEWS

The question of tropisms

The immediate responses of organisms to stimulating changes in their environment have claimed little attention from American investigators of plant physiology, and perhaps rightly so, since the interpretation of such phenomena is fraught with so much difficulty. The study of developmental responses under controlled conditions appeals to many as offering better opportunity for the advancement of knowledge of plant behavior. Possibly we have neglected immediate response physiology more than we should. At any rate, animal physiologists and students of behaviorism have made up for some neglect on the plant side.

A monograph by ROSE¹ summarizes this field of physiology for both the plant and animal kingdoms. The first part deals with the experimental facts, the second with general theories. The experimental survey falls into two sections, plant responses, and animal responses. The plant responses occupy 130 pages, and include tactic responses, geotropism, phototropism, and other plant tropisms. The section on animal responses, 227 pages, considers phototropism, galvanotropism, geotropism, and other animal tropisms. The theoretical part discusses JENNINGS' trial and error theory of response, LOEB's mechanistic theory, which is critically examined, and tropisms and general culture. This final chapter deals with tropisms in the general physiology of organisms, relation of tropisms to habits, instincts, development, immunity, etc., and their bearing on the problems of psychology and philosophy. Extensive bibliographies have been compiled which will be valuable to any one seeking a broad introduction to the difficulties of this field, with its many unsolved problems.--C. A. SHULL.

Revised edition of the Chicago textbook

At the time of the first appearance of Volume III of the Chicago textbook, twenty years ago, much surprise and not a little disappointment was expressed in many quarters that this, the first comprehensive textbook of plant ecology, should deal almost wholly, not with the ecology of vegetation but with the ecology of plants as individuals. The judgment of the author has been more than justified, however; for it has become increasingly evident, in the light of subsequent studies, that the structure and phenomena of vegetation can be satisfactorily interpreted only in the light of a complete understanding of the structure and behavior of the plants which collectively go to make up the vegetation.

¹ ROSE, MAURICE, *La question des tropismes*. 8vo. pp. vii+469. Les Presses Universitaires de France. 1929.

The original publication, by COWLES, has been thoroughly revised and brought up to date by FULLER.² As might be expected, not only have there been considerable alterations in the subject matter throughout, but a number of new topics have been introduced; such, for example, as transpiration and xeromorphic leaf structure, life forms of stems and the biological spectra of RAUNKIAER, and photoperiodism. The brief chapter on plant associations, now "plant communities," has been considerably modified. But perhaps the most surprising feature, in comparing the new with the old, is the comparatively small amount of alteration which has been necessary in order to bring the text completely into accord with modern ecological thought. Other books on plant ecology have been published, but the present volume remains unique in focusing attention on the ecological relations of plants as individuals.—G. E. NICHOLS.

Plant communities of the world

A volume³ has recently appeared which is the outgrowth of an earlier publication,⁴ in which the present author, collaborating with BROCKMANN-JEROSCH, proposed a classification of the plant communities of the world based on physiognomy and ecological structure. The framework of the present treatment remains the same, with certain modifications of detail. Three principal vegetation types are recognized, namely, *lignosa* or woody vegetation, *herbosa* or herbaceous vegetation, and *deserta* or desert vegetation; with a fourth and somewhat anomalous type, *phytoplankton* and *phytocedaphon*. Under *lignosa* are distinguished seven formation classes (e.g., *pluvilignosa* or rain woodland, *aestilignosa* or broadleaf deciduous woodland of temperate regions, *hiemilignosa* or broadleaf deciduous woodland of tropical and semi-tropical regions); and each of these in turn is divided into two or more formation groups (e.g., rain forest, rain scrub, etc.). As in the earlier work, concise descriptions are given of the various vegetation types, formation classes, and formation groups, but the present work goes much further. Not only are these generalized descriptions amplified, with copious data regarding climatic relations, etc., but descriptions are also given of the specific plant formations by which the larger vegetation units are represented in different parts of the world. The text is well organized and admirably written, in very readable German. It is profusely illustrated and accompanied by an excellent colored vegetation map. On the whole, RÜBEL's book is by far the most satisfactory general account of the earth's vegetation that has yet been published.—G. E. NICHOLS.

² COULTER, J. M., BARNES, C. R., and COWLES, H. C., A textbook of botany Vol. III. Ecology. Revised and enlarged by G. D. FULLER. 8vo. pp. x+498. Figs 541. American Book Co. New York. 1931. \$2.80.

³ RÜBEL, E., Pflanzengesellschaften der Erde. 8vo. pp. viii+464. Figs. 242, map. Hans Huber. Bern-Berlin. 1930.

⁴ BROCKMANN-JEROSCH, H., and RÜBEL, E., Die Einteilung der Pflanzengesellschaften nach ökologisch-physiognomischen Gesichtspunkten. 8vo. pp. iv+72. Fig. 1. Wilhelm Engelmann. Leipzig. 1912.

Tree mycorrhiza

An English translation⁵ of a book which summarizes MELIN's notable contributions to our knowledge of tree mycorrhiza is most welcome. It puts in compact and available form a mass of information that has accumulated during the past two decades. The present day knowledge of the nature of the root fungi of trees is reviewed, the growth of root fungi in pure cultures is described, the methods used in growing sterile cultures of tree seedlings are noted, and the physiology of symbiosis of the fungi and trees discussed. There is also a consideration of the possibility of nitrogen assimilation by the fungi. Among the trees that have been studied by MELIN are *Pinus silvestris*, *P. montana*, *Larix europa*, and *Picea abies*; and notable among their fungal symbionts are *Boletus badius*, *Boletus* spp., *Amanita muscaria*, *Lactarius deliciosus*, *Russula fragilis*, and several species of *Tricholoma*. The value of the book is increased by the addition of a good bibliography.—G. D. FULLER.

Glucosides

The glucosides are widely distributed in plants, yet their functions are but poorly understood. DIETERLE⁶ is the author of the second edition of VAN RIJN's *Chemical monograph of the plant glucosides*, the first edition of which was published in 1900. In the second edition only the naturally occurring glucosides are included. An attempt is made to give an exhaustive treatment of the subject. The glucosides are arranged according to family, those of 92 plant families being considered. In so far as possible, there is included in regard to each glucoside information concerning methods of preparation, identification and synthesis; characteristics, formula, and physiological effects. The book is well supplied with references, these appearing as footnotes. It will be welcomed by botanists as a valuable reference book. It should also be of use to animal physiologists, medical investigators, and all workers interested in the medicinal uses of glucosides.—S. V. EATON.

⁵ MELIN, ELIAS, Investigations of the significance of tree mycorrhiza: an ecological-physiological study. Translated from the German by PAUL W. STICKEL. pp. 173. Figs. 48. Mimeographed. Edwards Brothers. Ann Arbor, Michigan. 1930:

⁶ VAN RIJN, J. J. L., Die Glykoside. 2d ed. by H. DIETERLE. pp. vi+620. Gebrüder Borntraeger. Berlin. 1931.

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